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PAGET'S DISEASE OF THE NIPPLE

WITH SPECIAL REFERENCE TO THE CHANGES IN THE DUCTS *

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In 1936 I published a monograph[1] entitled "Paget's Disease of the Nipple and Its Relation to Surface Cancers and Precancerous States in General." Since then I have examined other examples and, while the results in the main have been confirmatory of my previous opinions, they call for certain modifications of my earlier views.

THE NATURE, ORIGIN, AND MODE OF SPREAD OF PAGET'S DISEASE

My opinions under this heading are represented diagrammatically in Text-Figure 1. Paget's disease is neoplastic from the outset. The initial lesion is a duct cancer arising from lining epithelial cells *in situ*. This primary growth arises in one or more ducts near their outlets (Text-Fig. 1, duct 1,A). This primary duct cancer extends from its site of origin (Text-Fig. 1, duct 1,A) by continuous intra-epithelial spread downwards in the epithelium lining the duct wall to the acini, and upwards and outwards into the epidermis where it causes the cutaneous lesion. From the intra-epithelial extension in the epidermis the cancer spreads down neighboring ducts (Text-Fig. 1, ducts 2, 3, and 4) and pilosebaceous follicles (Text-Fig. 1,F) as in the duct primarily affected. At a later stage the cancer which has spread down a duct into the underlying breast, either directly (Text-Fig. 1, duct 1) or indirectly by way of the epidermis (Text-Fig. 1, duct 2), breaks through the wall of the duct (Text-Fig. 1, duct 1,G, or duct 2,H) or acinus, and infiltrates the connective tissue of the breast. There is thus a link between the primary ductal growth (Text-Fig. 1, duct 1,A) and the infiltrating cancer (Text-Fig. 1, duct 1,G, or duct 2,H) in the underlying breast.

In Paget's disease the cancer spreads down the duct, not in the form of a solid column (as in permeation of lymphatic vessels) but as a hollow cylinder (Text-Fig. 1, duct 4,E); speaking generally, however, the cancer, as it spreads down the duct, disintegrates the lining epi-

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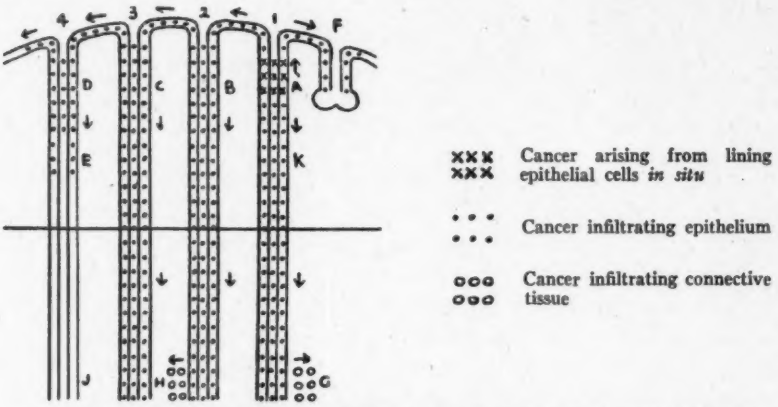
thelium of the duct and cancer cells occupy the lumen (Text-Fig. 1, ducts 1,K; 2,B; 3,C; and 4,D). In Paget's disease the duct cancer which results from centrifugal spread from the site of origin is a special variety and is essentially different from ordinary duct cancer. It is not suggested that there is anything special about the cancer cells; the special quality is in the intra-epithelial mode of spread. The epidermal lesion and the special duct cancer of Paget's disease are essentially the same; minor histological differences can be accounted for by variation in anatomical situation; both are examples of cancer spreading by the intra-epithelial route.



Text-Fig. 1. Diagrammatic representation of possible sequences of events in Paget's disease of the nipple. Continued in Text-Figures 2 to 4.

In Paget's disease the duct changes due to intra-epithelial spread may be simulated by a newgrowth arising from duct epithelium *in situ* when such a primary growth does not show the formation of papillomatous processes; further, just as Paget's disease of the skin of the nipple is simulated microscopically by cancer which has spread from the connective tissue of the breast directly into the epidermis (Text-Fig. 4,W), so the special duct cancer of Paget's disease is simulated when a cancer which is infiltrating the connective tissue of the breast breaks into ducts which, until then, have been free from growth (Text-Fig. 4, duct 3,T).

Ordinary Duct Cancer and Special Duct Cancer of Paget's Disease

There are three outstanding differences between ordinary duct cancer and the special duct cancer of Paget's disease.

In the ordinary duct cancer: (1) The growth arises as a result of the proliferation of lining epithelial cells *in situ*. (2) The ducts show

a variety of changes, and all stages of transition may be seen from simple papillomatous growth to masses of undifferentiated cells, that is, from a precancerous to a cancerous state. (3) The neoplastic proliferation within the ducts may occur in multicentric foci of origin, not only in different ducts, but also in different parts of the same duct.

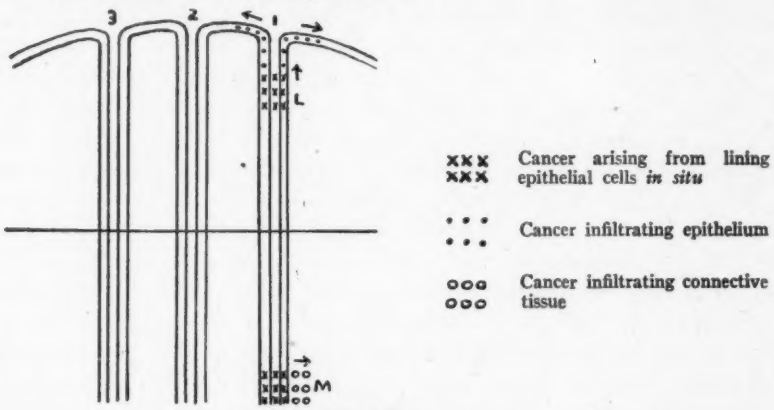
In the special duct cancer of Paget's disease: (1) The growth does not arise from the lining epithelial cells *in situ;* the cancer cells have infiltrated from a distance as a part of a centrifugal spread by the intra-epithelial route from the primary lesion. (2) The growth is one of full-fledged cancer; no precancerous or transitional stages are to be seen. (3) There are no separate foci of origin.

Duct cancer spreading by the intra-epithelial route (special duct cancer of Paget's disease) is not necessarily limited to Paget's disease, but it is the distinctive feature of the duct changes in Paget's disease, just as the intra-epithelial cancerous extension in the epidermis is the distinctive feature of the skin lesion in Paget's disease. In studying the early changes in the ducts in Paget's disease of the nipple great difficulty is experienced in distinguishing between those portions of the lesion in which there is neoplastic overgrowth *in situ* of the epithelial cells lining the duct, and those portions where the duct changes are all due to intra-epithelial spread of cancer.

It is possible that the intraductal growth arising from the lining epithelial cells *in situ* may assume a variety of forms or that all stages from a precancerous to a cancerous state may be present; or that it may have multicentric foci of origin in one or more ducts near their outlets; or that it may even dominate the picture so far as the ducts are concerned, the intra-epithelial spread in such cases being more extensive in epidermis than in ducts. In my experience, however, the initial ductal lesion arising from the lining epithelial cells *in situ* is usually inconspicuous and difficult to identify with certainty. This difficulty in identification of the primary growth may result from a close structural resemblance between the primary growth and its intra-epithelial cancerous extension; or the difficulty may be due to the primary growth being masked or obliterated by its intra-epithelial cancerous extension; or the primary growth may be small and situated to one side of the nipple so that it is not included in the sections cut for routine examination, which are usually taken from the middle of the nipple. Even if in Paget's disease upward intra-epithelial extension of cancer is seen in the nipple close to the epidermis, this would not invalidate the theory that intra-epithelial spread of cancer down one or more ducts forms a link between the skin lesion and the infiltrating cancer in the underlying breast, because the primary growth in a duct may be sufficiently deeply placed for its cancerous intra-

epithelial extension in an upward direction to be so early that it would be separated from the epidermis by a portion of unaffected duct.

I should like to mention here that Ewing [2] quoted me as having found the ducts intact in eight cases. This is incorrect. In my monograph I said: "On eight occasions after a careful examination was made of the whole breast no scirrhous cancer was detected, the disease being limited to ducts and epidermis without any evident extension of growth into the connective tissues." In point of fact I have never seen a case of Paget's disease of the nipple in which the ducts were free from cancer, and in a large percentage of the cases which I have examined, cancer had already infiltrated the connective tissue of the underlying breast, so that Paget's disease of the nipple should always be regarded as of serious significance.



Text-Fig. 2

The view held by Muir [3] as to the relation of the epidermal lesion to the infiltrating cancer in the underlying breast is "that the underlying and causative condition of both Paget's disease and the accompanying cancer is intraduct carcinoma, that is, a condition in which malignancy is present within ducts but has not broken through their walls. . . . It may occur in any part of the duct system and it may arise in multiple independent foci. If it occurs in the ducts of the nipple it may spread to its epidermis, Paget's disease resulting; this is a rare occurrence. The common event is a break-through in some part of the breast with carcinoma as the result." Under the heading "The relation of Paget's disease to carcinoma of the breast," Muir says: "In the first place, discontinuity between intraduct disease at a higher level and that at a lower can frequently be traced. The disease in the nipple may, in fact, be completely cut off from that in the breast."

Text-Figure 2 is a diagram showing in duct 1 two independent foci

of duct carcinoma arising from the lining epithelial cells *in situ*. One of these foci is in the nipple at L and the other in the underlying breast at M. From the focus in the nipple, cancer has infiltrated the epidermis, Paget's disease resulting. From the focus in the underlying breast, the duct cancer has broken through the wall and infiltrated the connective tissue with carcinoma of the breast as the result. The disease in the nipple is completely cut off from that in the breast. In Text-Figure 2 two completely independent primary cancers are represented, one infiltrating connective tissue, the other infiltrating epithelium, whereas in Text-Figure 1 all of the diseased parts are linked up with one primary cancer (duct 1,A).

In studying any disease it is desirable to eliminate complicating factors and to examine lesions in a pure state, and at as early a stage of their development as possible. In studying Paget's disease, it is therefore desirable to concentrate on those cases which are uncomplicated by infiltrating carcinoma of the breast, and Muir's group of five cases [3] in which "intraduct carcinoma was confined to the nipple" are of special interest. Suppose that these breasts where the nipple only was involved had not been amputated; then judging by experience in other cases, a cancer infiltrating the connective tissue would have formed in the underlying breast at a later date in each instance. I suggest that this infiltrating cancer in the breast would have resulted from downward extension.

In regard to the order of appearance of the epidermal lesion and the infiltrating carcinoma in the underlying breast Paget,[4] in his original paper "On Disease of the Mammary Areola Preceding Cancer of the Mammary Gland," wrote: "I believe it has not yet been published that certain chronic affections of the skin of the nipple and areola are very often succeeded by the formation of scirrhous cancer in the mammary gland. I have seen about fifteen cases in which this has happened, and the events were in all of them so similar that one description may suffice." No mention is made in this paper of mammary cancer preceding the lesion of the nipple.

In only 1 of the 24 cases included in my published series [1] was there clinical evidence suggesting that the epidermal lesion appeared after the cancer had formed in the underlying breast. If, in Paget's disease associated with infiltrating carcinoma of the underlying breast, there are two separate and independent infiltrating cancers as represented in Text-Figure 2, it is surprising that the cancer in the underlying breast should not more often precede the cancer in the nipple (with its cutaneous extension).

In considering the question of discontinuity between an affected

part of a duct in the nipple and the disease deep in the breast, it is important to recognize the distinction between ordinary duct carcinoma and the special duct carcinoma of Paget's disease. In ordinary duct carcinoma there may be discontinuity with multiple foci of origin and various stages of activity, but in the special duct carcinoma of Paget's disease there is no true discontinuity. The question of discontinuity of the special intra-epithelial cancer of Paget's disease, whether in epidermis or in ducts, may be considered from three points of view:

1. Apparent discontinuity is common near the advancing margin of the lesion whether in epidermis or in ducts, and this apparent discontinuity is probably due to death of some of the cancer cells.

2. In the epidermal lesion apparent discontinuity may be due to irregular rate of growth at the advancing margin of the lesion; in some areas at the periphery of the lesion advance may be rapid, in others slow. Sections cut in certain planes through these areas may show zones of epidermis invaded by cancer separated by zones of epidermis free from cancer. In the cramped space in ducts there is less scope for such an arrangement of alternating cancerous and noncancerous areas than there is in the epidermis, but even in the ducts this may occur to a slight extent near the advancing margin of the lesion.

3. In the main body of the epidermal lesion there may be areas in which the epidermis is completely destroyed by the intra-epithelial cancer, these areas being superficial ulcers with floors formed of granulation tissue. In affected ducts there may be areas where there is no epithelium on the wall, and where the lumen is occupied by necrotic cancer cells. The point which calls for special emphasis, however, is that in the main track of the ductal lesion there is never a considerable length of duct lined by normal epithelial cells.

The special duct cancer of Paget's disease may be present in the upper part of a duct and ordinary duct cancer be present in the lower part of the same duct, the two affected parts being separated by a considerable length of normal duct. I suggest that this is the explanation of the following statement made by Cheatle and Cutler [5] in their chapter on Paget's disease of the nipple: "In describing other cases of this series ducts were also depicted in which neoplastic epithelial growth was limited to their upper parts and which, after long intervals of normal ducts, led into the epithelial neoplasia situated in their final distributions. In many of these terminal ducts were also papillomatous formations which may be assumed to be of primary spontaneous growth arising in the epithelium of those structures as the disease in the upper ducts was nonpapillomatous."

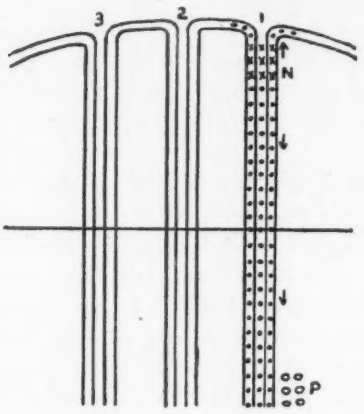
In some cases of Paget's disease the neoplastic overgrowth which constitutes the initial lesion may not be limited to a duct or ducts near their outlets, but may be present also in other separate foci in ducts in the underlying breast. Should this occur, the situation might arise where a focus of the initial neoplastic epithelial overgrowth in a duct near its outlet would have become cancerous and spread by the intra-epithelial route down that duct or (via the epidermis) down neighboring ducts. This spread down the ducts might have extended to different levels; in some it might have extended down to the acini (Text-Fig. 1, duct 3), in others it might have extended only a short distance down the ducts and so involved only their upper parts (Text-Fig. 1, duct 4). In the deeper parts of one or more of these ducts (Text-Fig. 1, duct 4,J), whose upper parts are the seat of full-fledged cancer spreading downwards by the intra-epithelial route, there might be separate foci of initial neoplastic epithelial overgrowth of the same order as that which became carcinomatous near a duct outlet (Text-Fig. 1, duct 1,A) and later infiltrated epidermis and ducts by intra-epithelial spread. This deep focus of initial neoplastic epithelial overgrowth (Text-Fig. 1, duct 4,J) might be at any stage of transition from a benign condition to duct carcinoma, and papillomatous formation might be one of the appearances seen early in the transition; a variety of stages in the transition might be present at any one time. In such a duct (Text-Fig. 1, duct 4) the upper part (D and E) would show the special duct cancer of Paget's disease, the lower part (J) would show ordinary duct cancer, and the two parts would be separated by a portion of the duct free from growth. Even if this discontinuity were present in some ducts, continuity of cancer spreading by the intra-epithelial route from the upper limits of ducts down to the acini might be present in other ducts (Text-Fig. 1, ducts 1, 2, and 3), so that infiltration of connective tissue deep in the breast might come from either ordinary duct cancer (Text-Fig. 1, duct 4,J) or from special duct cancer of Paget's disease (Text-Fig. 1, duct 1,G, or duct 2,H).

Possible Sequence When, as Occasionally Happens, Paget's Disease of the Nipple Is Preceded by Infiltrating Cancer in the Underlying Breast

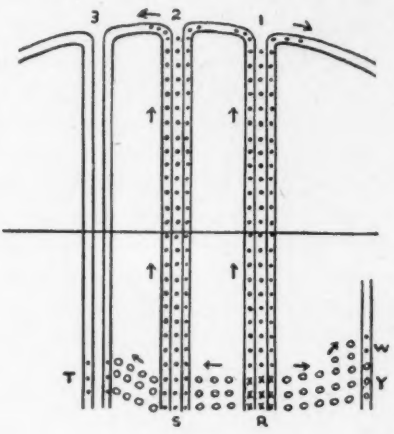
Text-Figure 3 represents what I suggested in my monograph [1] as the most likely sequence of events. A focus of duct cancer which has arisen from the lining epithelial cells *in situ* near the duct outlet is represented in duct 1 at N. This cancer has spread down the duct by the intra-epithelial route and broken through the wall of the duct in the underlying breast at P with infiltrating cancer as the result. Only after this

had occurred did the focus of duct cancer at N infiltrate the epidermis by the intra-epithelial route.

Another possible sequence of events is represented in Text-Figure 4. A focus of duct cancer which has arisen from the lining epithelial cells *in situ* in the underlying breast is represented in duct 1 at R. This cancer has broken through the duct wall and invaded the connective tissue of the breast. From the connective tissue the cancer has spread on the left into the deep part of duct 2 at S and duct 3 at T, and on



Text-Fig. 3



Text-Fig. 4

Cancer arising from lining epithelial cells *in situ*

Cancer infiltrating epithelium

Cancer infiltrating connective tissue

the right it has spread to involve the skin of the breast at W and Y. From the primary focus of duct cancer at R or from the secondarily invaded deep part of duct 2 at S cancer has spread upwards by the intra-epithelial route and entered the epidermis producing lesions essentially the same as that found in Paget's disease uncomplicated by infiltrating cancer in the underlying breast.

The reasons why the sequence of events represented in Text-Figure 3 was suggested in my monograph [1] as the most likely explanation when the infiltrating cancer in the underlying breast preceded the epidermal lesion are as follows:

1. In some cases of uncomplicated Paget's disease the epidermal lesion is very small and yet the ducts are extensively involved by cancer, both in the nipple and in the underlying breast, so much so that the appearances suggest that cancer might break through the duct

walls and infiltrate the connective tissue in the underlying breast at any moment. It is not going much further to suppose that this break-through might occur before there is infiltration of the epidermis by the primary duct cancer near the duct outlet.

2. No doubt in many infiltrating cancers of the underlying breast the cancer cells in the connective tissue irrupt into uninvolved ducts as in Text-Figure 4, duct 3,T, and intra-epithelial spread up the duct commences, but before this intra-epithelial cancer spreads far (as in Text-Fig. 4, duct 2), it is overwhelmed by the neighboring cancer spreading in the connective tissue. Similarly, when infiltrating cancer spreads through the connective tissue of the breast to involve the epidermis as at W in Text-Figure 4, cancer cells may at first enter the epidermis and produce appearances similar to those of the epidermal lesion in Paget's disease, but the rapid growth of the infiltrating cancer in the connective tissue destroys the skin and a malignant ulcer forms. Occasionally the cancer cells may spread a short distance in the squamous epithelium at the edge of the malignant ulcer, but they seldom spread far enough to produce a condition like that in the epidermis in Paget's disease. Dunn,[6] however, has described invasion of the cuticle by a mucinous carcinoma at the lower end of the external anal sphincter.

Ascending duct cancer in breasts where infiltrating cancer already exists has been described and illustrated by Muir,[3] but the evidence in his illustrations is, in my opinion, not altogether convincing; in particular his Figure 6 is, I think, open to another interpretation. The legend to this illustration is as follows: "Fig. 6. Intraduct carcinoma in duct of nipple, of older standing in lower part and just reaching the orifice. Case 2. × 18." Referring to his case 2 (from which his Figures 6 and 7 were taken), Muir said: "Extensive intraduct carcinoma was present in the nipple and breast, also infiltrating cancer in the breast and in the lower part of the nipple. One duct in the nipple was completely filled with Paget cells right up to the orifice. . . ." The duct in the middle of Muir's Figure 6, presumably the one which he said was completely filled with Paget cells right up to the orifice, contains no cells in its lumen in the lowest part in this illustration, and the duct wall in this situation appears free from growth. This appearance is against spread of cancer up the duct from a lower level. The small duct shown on the left of this figure is not referred to by Muir, but it appears to be the seat of cancer, especially in the upper part. As this duct is traced downwards it is seen to break up into what look like acini, some of which seem to be free from cancer. In this duct the cancer seems older in the upper part than in the lower part, the

appearances, in my opinion, being in favor of downward rather than of upward spread of cancer. These comments notwithstanding, I consider that when Paget's disease of the nipple is preceded by infiltrating cancer in the underlying breast, either of the sequences of events represented diagrammatically in Text-Figures 3 and 4 may have occurred.

Illustrative Cases

Case 1

The breast (P.U.S. 2332) was amputated because of a "sore" which had been present on the nipple for 6 months. There was no pain and no discharge. Immediately before operation the nipple was slightly redder than normal, and a scab was adherent to its summit. No other abnormality was detected, and the axillary lymph nodes were not enlarged. Simple amputation of the breast was carried out.

The nipple was bisected vertically, and serial sections were cut from each half. (The serial sections, however, did not include the entire nipple.) In addition, blocks of tissue were taken from the underlying breast, and in four of these cancer was present within ducts, or within ducts and acini, but no convincing evidence of cancerous invasion of the connective tissue of the breast was found.

The appearance of a vertical section cut through the middle of the nipple is to be seen in Figure 1. The area of skin involved is small; this area is easy to define because of the zone of inflammatory cells in the dermis. Just to the left of the center of the illustration a large duct, involved throughout its whole extent, presents a striking feature. To the right of this duct are three others all affected in their upper parts but otherwise free from growth. The transverse clefts about the level of the base of the nipple are artifacts. On the right-hand side, deep to the nipple, are some slightly dilated ducts free from growth. To the left of these, and at about the same level, is an ill-defined dilated duct affected by growth throughout. Still farther to the left, at this level, is a clear area (adipose tissue) in which a large blood vessel is conspicuous.

In Figure 2 the portion of Figure 1 enclosed within the rectangle is shown more highly magnified. The upper parts of several ducts are included. That on the left is extensively invaded above and free from growth below. That in the middle is free from growth except at the extreme upper limit which is involved by growth, though this cannot be appreciated in the photomicrograph. To the right is a duct which is involved by growth throughout the whole extent above the transverse strip of connective tissue (which includes some involuntary muscle). The duct below this strip of connective tissue (probably a continuation of that above the strip) shows very early involvement in its uppermost part, and abundant epithelial cells in the lumen. The small

section of a duct, which looks like an inverted V, in the uppermost part of the illustration has cancer cells among the lining epithelial cells, though this cannot be appreciated in the photomicrograph.

The changes in the affected epidermis and ducts in this and other vertical sections of the nipple near its midline are akin, and can be understood by studying Figures 3 to 10 inclusive. Let us examine the epidermal lesion at an early stage (Fig. 3). Here we see cancer cells infiltrating the epidermis in its deeper part; to the left a cancer cell is to be seen separated by an appreciable distance from others that are evident as the cluster of cancer cells on the right is approached. It is not necessary to assume that the cancer cells wander in the epidermis; it is likely that many of the cancer cells die early, and that the apparently normal squamous epithelium between the cancer cells has actually been traversed by the growth. Another possibility is that continuity between the cancer cell extensions would be found in other planes of section.

When a more advanced stage of the epidermal lesion is examined, changes of longer standing in the epidermis are met with. These vary considerably in appearance. In some parts (Fig. 4) the surface of the epidermis remains intact and invading cancer cells are arranged in masses separated by thin layers of epidermis. In other situations the individual cancer cells are separate, and as a rule are more numerous in the deeper part of the epidermis, though sometimes they are scattered throughout its substance. In still other situations the epidermis has been destroyed by the growth, the condition being one of superficial ulceration, vascular connective tissue forming the floor of the ulcer. In spite of minor differences in histological characters, the epidermal lesion is fundamentally the same throughout, and is due to intra-epidermal invasion by carcinoma.

When the lesion is examined in affected ducts near their outlets the appearances in the duct and in the epidermis are essentially the same. Figure 5 shows the upper limits of three ducts opening together on the free surface of the nipple. In this plane of section only one of the ducts can be seen extending deep into the nipple. The resemblance of the ductal lesion to the epidermal lesion is especially close near the outlet of the duct, because the lining is here composed of squamous epithelium. As the duct is traced downwards, appearances like those in Figure 10 are to be seen. There is, however, considerable variation in the compactness or looseness of arrangement of the cancer cells in the affected ducts.

The special form of duct carcinoma present in ducts affected by Paget's disease is best illustrated by Figures 6, 7, and 8. In Figure 6

the upper part of the duct is affected, the lower part is free from growth; the affected portion is involved continuously down to the advancing margin. Near the advancing margin the growth is seen to be spreading in an intra-epithelial fashion; the cancer is spreading down the duct not as a solid column but as a hollow cylinder. A similar appearance is evident near the advancing margin of cancer in the duct illustrated in Figure 8. The part of Figure 6 indicated by the letter A is shown more highly magnified in Figure 7 in which the lining epithelium is to be seen resting on connective tissue to the left. The lining epithelial cells, which have small, darkly stained nuclei, are separated into two thin layers: an intact layer on the free surface, and an irregular and incomplete deeper layer near the connective tissue. The separation is caused by a zone of cancer cells with large, rounded or oval nuclei staining more faintly than the nuclei of the lining epithelial cells. In many instances a darkly staining nucleolus can be seen near the center of the nucleus of a cancer cell. The cytoplasm of the cancer cells is fairly voluminous and stains faintly. The duct changes in Figure 7 are considered to be essentially the same as the epidermal changes in Figure 4.

Figure 8, which shows a different duct from that in Figure 6, illustrates the earliest stage of the special form of duct carcinoma of Paget's disease. The lowest limit of extension of the cancer down this particular duct is included in this figure. The duct wall at the bottom of the illustration is free from growth. The duct changes in Figure 8 are considered to be essentially the same as the epidermal changes in Figure 3. Two conspicuous isolated cancer cells are to be seen on the left of Figure 8 situated in the epithelial lining. Higher up the duct, on both sides, there are more numerous cancer cells in the epithelial lining. At the top of the illustration, the cancer cells are more abundant and have broken through the lining epithelium, which has been obliterated or mixed up with the cancer cells occupying the lumen in this situation. The cancer cells have not come from the epithelial cells lining this duct at this level, but are descended from the cells of the initial cancerous focus from which they have spread by the intra-epithelial route. Just above the lower of the two isolated cancer cells on the left of Figure 8 is an ill-defined, rather large cell which may be a degenerate cancer cell, and just below the upper of the two isolated cancer cells on the left of Figure 8 is another ill-defined cell which may be a degenerate cancer cell. The appearances are consistent with the opinion that the cancer traversed the epithelium between these two apparently isolated cancer cells but that some of the cancer cells have

died. Where the cancer insinuates its way down the duct by the intra-epithelial route, appearances like those shown in Figures 6 to 8 inclusive are met, but quite commonly the intra-epithelial spread down the duct is more rapid or destructive with the result that the presence of individual cancer cells in relatively intact lining epithelium is not to be seen.

Figure 9 shows appearances corresponding closely with those in Figure 8 except that below and to the right in Figure 9 there is a focus of cancer apparently isolated and separated by uninvolved lining epithelium from what looks like the advancing margin of the duct cancer on the left. This apparently isolated focus is regarded as part of the intra-epithelial spread. It is exceptional to find such a wide gap of uninvolved lining epithelium in duct cancer spreading by the intra-epithelial route. In this particular instance the uninvolved lining epithelium between the advancing cancer on the left and the focus of cancer on the right may have been traversed by the cancer, but sections in other planes seem to show a link between the apparently isolated focus on the right and the collection of cancer cells on the left which are just short of the advancing margin in this situation.

An important point that must be stressed is that any semblance of discontinuity in cancer extending by the intra-epithelial route is limited to the region where the cancer is actually spreading; once the cancer reaches the stage represented in the upper parts of Figures 6, 8, and 9 or throughout the whole of Figure 10, then normal lining epithelium is destroyed. It is true that the cancer cells in an affected duct may undergo necrosis and that no live cells may be present either in the lumen or on the wall of an involved duct at certain levels, but there will not be a return to an appearance resembling that of a normal duct.

The later stage of the ductal lesion is well shown in Figure 10, and this is the appearance which is usually such a striking feature of the ducts in Paget's disease of the nipple. The changes are essentially the same as those in the epidermal lesion (Fig. 4), but in the epidermis the squamous epithelium is firmer and not so easily disintegrated as is the epithelium which lines ducts.

In Figure 10 the duct is occupied by cancer cells which have no special arrangement. There may be a few of the original lining epithelial cells mixed up with the cancer cells, but in such older parts of the ductal lesion the cancer cells dominate the picture. Sometimes the lining epithelial cells are abundant in the lumen near the advancing margin of the ductal lesion and slightly beyond this advancing margin;

this will be referred to later (see Fig. 18). If the irregular arrangement of the cancer cells in the upper parts of the ducts illustrated in Figures 6 and 8 is compared with the appearances shown in Figure 10, it will be seen that there is a close resemblance between them. In this particular breast all of the affected ducts except A in Figure 12 show appearances which seem to be essentially the same as those illustrated in Figure 10; that is to say, they show these appearances where the ductal lesion is well established, but at the advancing margin the appearances are like those shown in Figures 6 and 8. In an occasional affected duct which resembles that illustrated in Figure 10 there is a suspicion of glandular arrangement, the cancer not being completely undifferentiated.

It is recognized that appearances like those illustrated in Figure 10 may result from cancer arising *in situ* from epithelial cells lining ducts, and a definite opinion to the contrary could not be given if attention were paid to this area alone, but the appearances in Figure 10 are essentially the same as those in the upper parts of Figures 6 and 8 and it is thought that the evidence is strong that in Figures 6 and 8 we are dealing with the special form of duct cancer characteristic of Paget's disease.

Figure 11 shows a duct leading into acini; this duct is in one of the blocks of tissue taken from the underlying breast. In Figure 11 duct and acini are involved in cancer throughout. Much of the growth in the duct is necrotic. The isolated groups of epithelial cells to the left of the illustration are thought to be acini invaded by cancer.

It is thought that when duct cancer spreading downwards by the intra-epithelial route as illustrated in Figures 6 and 8 continues its downward extension, duct changes like those shown in Figure 10 result; and that when the downward spread extends to the acini, changes like those shown in Figure 11 result. That is the interpretation placed on Figure 11 though it is recognized that, taken purely on the evidence revealed by Figure 11, the changes could be due to neoplastic overgrowth of lining cells *in situ*.

The epidermal and duct changes which have been described so far (Figs. 1 to 11) are those which I regard as characteristic of Paget's disease of the nipple. In all of these ducts, and in the epidermis, the lesion is one of full-fledged cancer; there is no recognizable starting point, and no precancerous change is to be seen. In many cases, for example case 2, these changes, or others essentially like them, are all that are to be found in an ordinary routine examination of a breast affected by Paget's disease.

The description up to this point (except that related to Fig. 11) applies to sections near the midline of this nipple. When serial sections farther and farther away from the midline are examined, a section is reached where, in addition to ducts showing the characteristic changes already described, there is a duct showing different changes—changes which suggest that possibly the original neoplastic lesion started in this particular duct. This duct showing special features (along with many other ducts showing the features already described) is to be seen in the section illustrated in Figure 12. In this illustration some ten ducts are present; serial sections show, however, that what appear to be separate ducts are in some instances different portions of the same duct. All of the ducts in this illustration show similar histological appearances except the one marked A. After studying the serial sections, the impression was gained that though duct A is somewhat irregular in its distribution, it has two or three main branches. Apparently isolated groups of cancer cells in this and other situations in the nipple, as well as in the underlying breast tissue, might suggest that there is infiltration of connective tissue, but when serial sections are examined, many of these apparently isolated groups of cancer cells are seen to link up with ducts and acini of which they represent outlying parts. No convincing evidence of infiltration of the connective tissue was found in this breast, either in the nipple or in underlying mammary substance.

In duct A of Figure 12 there is neoplastic tissue with a distinctly glandular structure. This is well shown in Figure 13 which is not taken from the same section as Figure 12 but from a section close to it in the series. When duct A is traced downwards in the serial sections, the glandular cancer is seen to extend into the upper ends of the branches of this duct for a short distance, and then to merge insensibly in undifferentiated cancer which extends downwards by the intra-epithelial route as in the other affected ducts in this breast. When duct A is traced upwards in the serial sections, the glandular structure of the growth is evident almost to the level of the epidermis, but in the epidermis the growth shows no evidence of glandular structure. (Occasionally in Paget's disease the cancer does present a tendency to glandular structure even in the epidermis.) The appearances of the neoplastic tissue in Figure 13 suggest origin from the epithelium *in situ*. Even though the arrangement is fairly regular, the appearances are thought to be consistent with the growth being cancerous.

A striking contrast with duct A (shown also in Fig. 13) is presented by the other ducts in Figure 12, all of which are alike except that

some are involved by growth throughout, whereas others (B, C, D, E, F) are affected only in their upper parts. The five ducts affected only in their upper parts are shown more highly magnified in Figures 14 to 18 inclusive which are all taken from the same section as Figure 12. The changes in all of the ducts in Figure 12 (except A which is shown also in Fig. 13) are essentially the same as those illustrated in Figures 6 and 8, and are consistent with duct cancer spreading downwards by the intra-epithelial route. In some of these ducts, and especially in that illustrated in Figure 18, the lumen of the duct contains abundant epithelial cells well below the lowest limit of extension of the cancer. In this situation large numbers of the cells in the lumen are nonneoplastic epithelial cells thrown off the wall as a result of the cancerous invasion, though no doubt cancer cells are mixed with the nonneoplastic cells. As the cancer spreads down the duct, the cells which occupy the lumen where the lesion is fully established are practically all cancer cells.

While recognizing that cancer may spread by the intra-epithelial route and yet present a glandular structure, still the contrast between the structure of the growth in the upper part of duct A (Figs. 12 and 13) and that of all the other affected ducts in the breast is so striking that the following suggestion is submitted as a possible explanation of the sequence of events in case 1.

Possible Sequence of Events in Case 1

If we assume that the opinions already expressed concerning the changes in the epidermis and in the ducts are correct, then we have the following information to guide us:

1. Intra-epidermal cancer spreading by the intra-epithelial route is present in the epidermis on the summit of the nipple.
2. A large number of ducts in the nipple are the seat of duct cancer.
3. All but one of these affected ducts contain undifferentiated cancer.
4. The ducts showing undifferentiated cancer are to be seen at all levels in the nipple and also in the underlying breast.
5. In the underlying breast the undifferentiated cancer in ducts extends into acini.
6. Where ducts containing undifferentiated cancer are seen in particular sections to be affected in one part and not in another, it is invariably the upper part that is affected and the lower part that is unaffected.
7. In such ducts where the upper part is affected and the lower part is unaffected, the zone where these two parts meet shows spread

of the cancer downwards in the epithelial lining towards the uninvolved lower part of the duct.

8. When the uninvolved lower parts of such ducts are traced downwards in serial sections they are seen to be free from growth.

9. When the involved upper parts of such ducts are traced upwards in serial sections they are seen to be continuously involved up to the epidermis.

10. When such ducts are traced into the epidermis the changes in the ducts are continuous with the changes in the epidermis.

11. The changes in these ducts appear to be essentially the same as the changes in the epidermis, both at the advancing margin of the lesion and in those parts of the lesion which are more advanced and of longer standing.

12. The one affected duct which does not contain undifferentiated cancer contains cancer with a glandular structure (duct A of Figs. 12 and 13). The glandular cancer is situated in duct A high in the nipple. When this glandular cancer is traced upwards in duct A its glandular character disappears, and in the epidermis the cancer is all undifferentiated. When traced downwards this glandular cancer extends into the commencement of the branches of duct A but then merges insensibly in undifferentiated cancer which extends farther down the branches.

With this information before us the following suggestion is made as to the sequence of events in case 1. The disease commenced in this breast in one duct (duct A of Figs. 12 and 13) situated not in the middle of the nipple but somewhat towards one side of the nipple. The cancer arose from the lining epithelial cells *in situ* and assumed a glandular structure; it involved this duct from almost the level of the epidermis down to and including the upper ends of its main branches. This length of duct is regarded as the site of origin of the primary cancer. When the primary cancer in duct A extended from its site of origin downwards in the branches of duct A and upwards and outwards in the epidermis, it lost its glandular structure and spread as an undifferentiated cancer by the intra-epithelial route, no longer arising from the lining epithelial cells *in situ*. From the epidermis the undifferentiated cancer spread by the intra-epithelial route down many other ducts. In these ducts the downward extension reached various levels and in some of them it spread into the mammary tissue deep to the nipple.

In relation to Text-Figure 1 it is thought that the ducts in Figure 2 correspond with Text-Figure 1, duct 4; Figure 5 with Text-Figure 1,

duct 3 or duct 4,D; Figures 6, 7, 8, and 9 with Text-Figure 1, duct 4,E; Figure 10 with Text-Figure 1, duct 3 or duct 4,D; Figure 11 with Text-Figure 1, duct 3; Figure 12, duct A (Fig. 13) with Text-Figure 1, duct 1,A; Figure 12, ducts B (Fig. 14), C (Fig. 15), D (Fig. 16), E (Fig. 17), and F (Fig. 18) with Text-Figure 1, duct 4; and the remaining ducts in Figure 12 with Text-Figure 1, duct 3 or duct 4,D. In case 1 no break through the duct wall as in Text-Figure 1, duct 1,G, and duct 2,H, had occurred.

In case 1, intra-epithelial spread down pilosebaceous follicles is not to be seen and very early intra-epithelial spread down ducts is not striking, but these are both conspicuous in case 2.

Case 2

The patient (P.U.S. 2677) was an unmarried woman, 56 years of age, who said that a small eroded area had appeared on the right nipple 4 months before the breast was amputated. The eroded area had gradually spread to involve the remainder of the nipple and the whole areola. The nipple had always been retracted. Radical removal of the right breast and axillary lymph nodes was performed.

After fixation of the specimen the ulcer in the nipple area measured 2.5 cm. in diameter. The nipple was depressed. On bisecting the breast through the center of the nipple the skin in the nipple area was seen to be increased in thickness (maximum thickness, 0.7 cm.). In the mammary substance there was no cystic condition and no nodule of growth; indeed, the mammary tissue appeared relatively normal, though when it was cut it felt rather tough.

Microscopical examination revealed cancer in the epidermis of the nipple, also in ducts and acini of both nipple and underlying mammary gland, but no infiltration of the connective tissue was to be seen and none of the five axillary lymph nodes examined showed invasion by cancer.

Figure 19 shows epidermis infiltrated by cancer cells, some of which are large and some degenerate; the spaces are due to clefts between the cancer cells and the epidermal cells. In Figure 20 a single large cancer cell is present; its isolation is probably due to other cancer cells having died and disappeared. Figure 21 shows cancer spreading by the intra-epithelial route from the epidermis down into a pilosebaceous follicle. The lowest limit of spread on the right side of the duct at A is shown under high magnification in Figure 22 (A in Fig. 21 corresponds with A in Fig. 22). In Figure 22 the cancer cells in the upper part are in fairly good condition, but those in the lower part are degenerate, some being represented only by small dark-staining fragments.

Figure 23 shows cancer in the epidermis extending down the wall of a duct. Figure 24 shows the continuation of the same duct. Figure 24 was not taken from the same section as Figure 23 but from one near it in the series. Taken in conjunction, Figures 23 and 24 show appearances consistent with the opinion that the cancer has spread continuously by the intra-epithelial route from the epidermis down to the lower part of the duct in Figure 24. Figure 25 shows appearances consistent with the opinion that cancer in the epidermis has spread by the intra-epithelial route into the uppermost part of an accessory duct. Figure 26 includes the commencement of a main lactiferous duct which is regarded as showing a later stage in secondary cancerous involvement due to cancer spreading by the intra-epithelial route from the epidermis down the duct, and later disrupting the epithelial lining of the duct and setting cancer cells free in the duct lumen. Changes like those illustrated in this figure are present in ducts deeper in the nipple and also in the underlying breast, but no appearances suggesting a primary focus were detected anywhere in the sections of nipple and underlying breast which were examined.

In relation to the diagrammatic representation in Text-Figure 1 it is thought that Figures 21 and 22 correspond with Text-Figure 1,F; Figures 23, 24, and 25, with Text-Figure 1, duct 4; and Figure 26 with Text-Figure 1, duct 3 or duct 4,D.

Summary and Conclusions

1. An account is given of changes that occur in Paget's disease of the nipple, with special reference to the changes in the ducts.

2. Two cases are reported in which these changes are illustrated.

3. A distinction is drawn between ordinary duct cancer in which the growth arises as a result of the proliferation of the lining epithelial cells *in situ* and the special duct cancer of Paget's disease in which the cancer cells have infiltrated from a distance as part of a centrifugal spread by the intra-epithelial route from the primary duct cancer which has arisen in a duct near its outlet.

4. In this special duct cancer of Paget's disease, the special quality is in the intra-epithelial mode of spread. It is not suggested that there is anything special about the cancer cells.

5. It is recognized that occasionally the nipple lesion and the mammary cancer may be two completely independent primary infiltrating cancers: that in the nipple, infiltrating epithelium; that in the underlying breast, infiltrating connective tissue.

6. Evidence is submitted, however, in support of the opinion that

usually in Paget's disease of the nipple the epidermal lesion precedes the infiltrating cancer in the underlying breast, and is linked to this infiltrating cancer by spreading down one or more ducts by the intra-epithelial route (special duct cancer of Paget's disease).

7. Points in this evidence are:

(a) Cases are met with at all three of the following stages: (1) involving only the epidermis of the nipple and ducts of the nipple; (2) in addition to (1), involving ducts and acini in the underlying breast; (3) in addition to (1) and (2), infiltrating the connective tissue in the underlying breast.

(b) In all of Paget's 15 cases and in all but 1 of the 24 cases in my published series,[1] the skin lesion preceded the cancer in the mammary tissue (as judged by clinical standards).

(c) Cases of Paget's disease uncomplicated by infiltrating carcinoma in the underlying breast provide microscopical evidence of intra-epithelial downward spread of cancer (special duct cancer of Paget's disease) to different levels in ducts and in pilosebaceous follicles.

(d) In some of the ducts where the evidence in support of downward spread of cancer is strong, the ducts look ripe for rupture which would result in cancerous infiltration of the connective tissue.

8. When, as occasionally happens, the infiltrating cancer in the underlying breast precedes the epidermal lesion, it is considered that there are two possible explanations:

(a) That the epidermal lesion is due to delayed intra-epithelial extension by a primary duct cancer arising from lining epithelial cells near the duct outlet, cancer having previously spread by the intra-epithelial route from the primary duct cancer down the duct into the underlying breast and broken through the duct wall in this situation with resultant infiltration of the connective tissue.

(b) That the epidermal lesion is due to irruption of cancer, infiltrating the connective tissue in the underlying breast, into a duct in this situation, with subsequent intra-epithelial spread up the duct into the epidermis.

9. In breasts amputated for Paget's disease of the nipple the skin lesion is always associated with duct cancer in the nipple. In the great majority of cases it is also associated with duct cancer in the underlying breast, and in a large percentage of cases cancer has already infiltrated the connective tissue of the underlying breast. Paget's disease, therefore, is always to be regarded as of serious significance.

I am indebted to the late Dr. Allan Gillies for providing me with the specimen from case 1 and to Dr. B. T. Edye for the specimen from case 2. I wish to thank Mr. L. Findlayson for technical assistance, Miss M. G. MacKenzie for secretarial assistance, and Mr. S. Woodward-Smith for drawing the diagrams and taking the photomicrographs.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 1

FIG. 1 (case 1). The cut surface of the nipple after bisection. × 4.

FIG. 2 (case 1). The portion of Figure 1 included in the rectangle is here shown more highly magnified. × 30.

FIG. 3 (case 1). The epidermal lesion at an early stage. × 275.

FIG. 4 (case 1). The epidermal lesion at a later stage. × 250.

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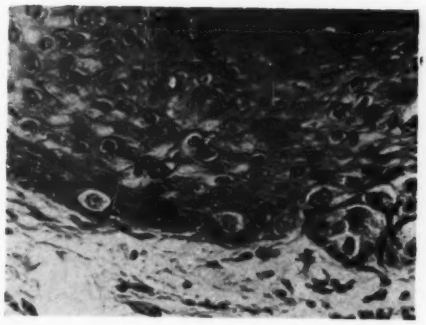

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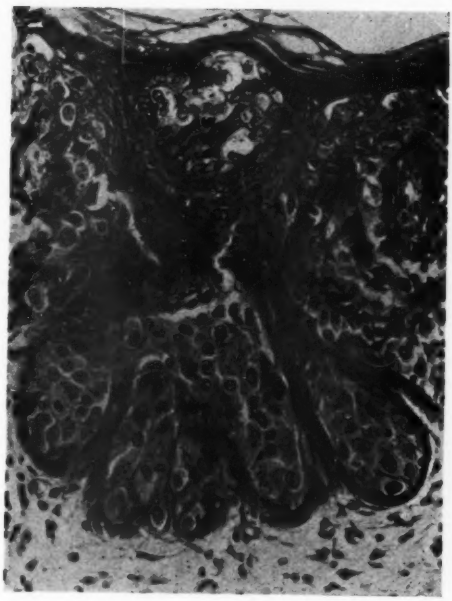

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Inglis

Paget's Disease of the Nipple

Plate 2

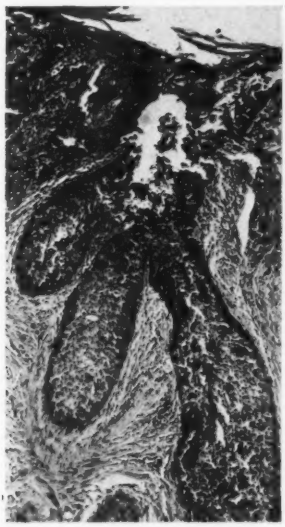
Fig. 5 (case 1). Cancerous ducts joining the epidermis. × 60.

Fig. 6 (case 1). Special duct cancer spreading downwards. × 50.

Fig. 7 (case 1). The portion of Figure 6 indicated by A is here shown more highly magnified. × 450.

Fig. 8 (case 1). The advancing margin of special duct carcinoma spreading downwards. × 275.

Fig. 9 (case 1). Special duct carcinoma showing apparent discontinuity at advancing margin. × 200.



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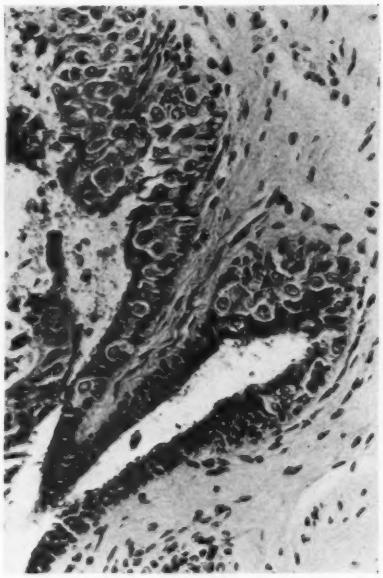

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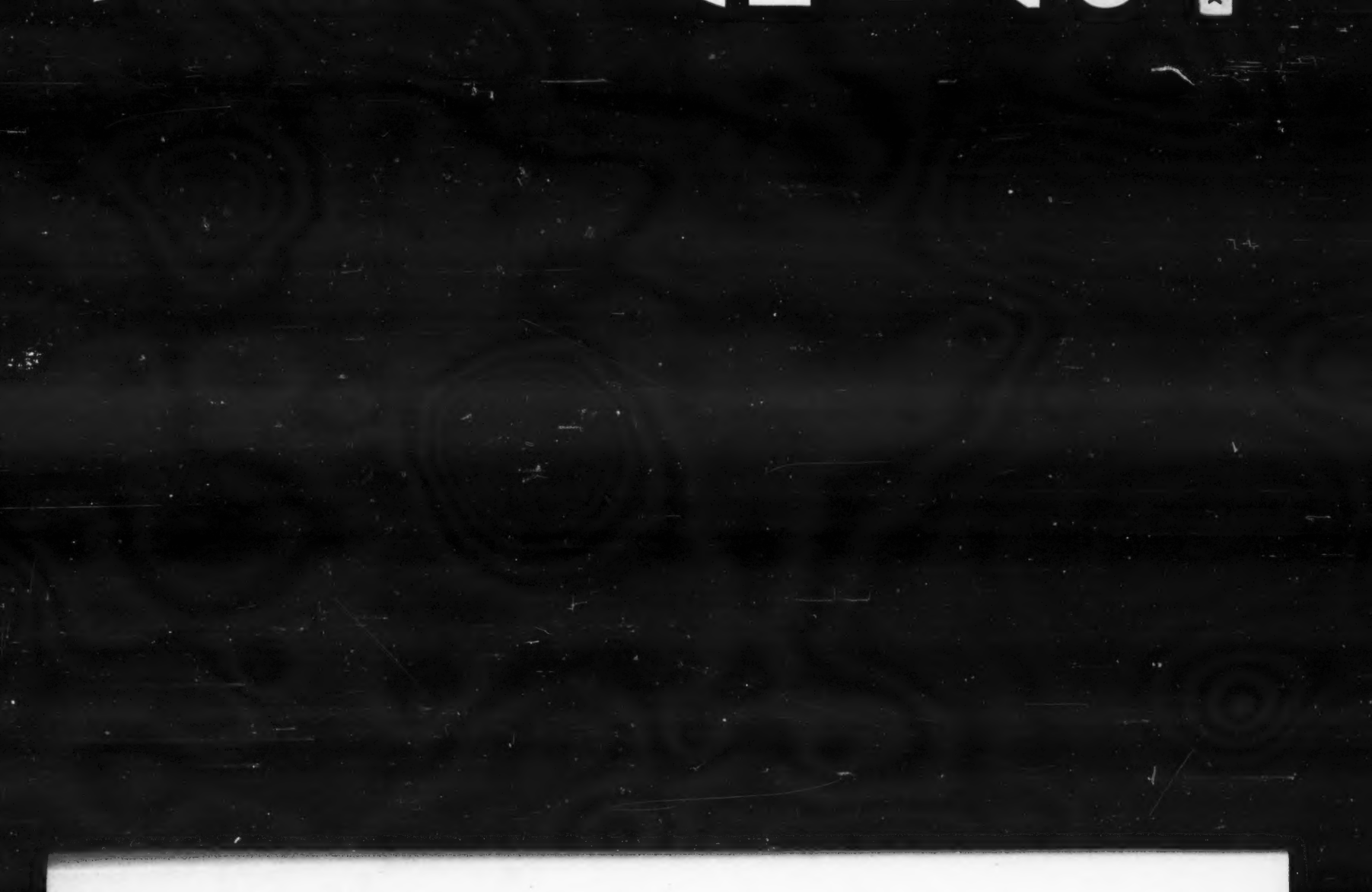
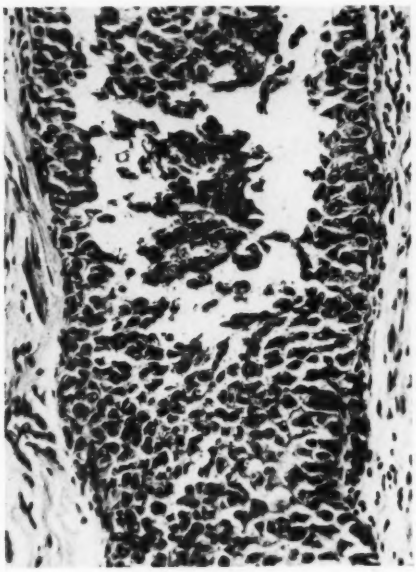
PLATE 3

FIG. 10 (case 1). Special duct carcinoma at an advanced stage. The appearances are also consistent with ordinary duct carcinoma. × 180.

FIG. 11 (case 1). Special duct carcinoma with involvement of acini. The appearances are also consistent with ordinary duct carcinoma. × 100.

FIG. 12 (case 1). A vertical section through the nipple some distance from the midline. × 9.





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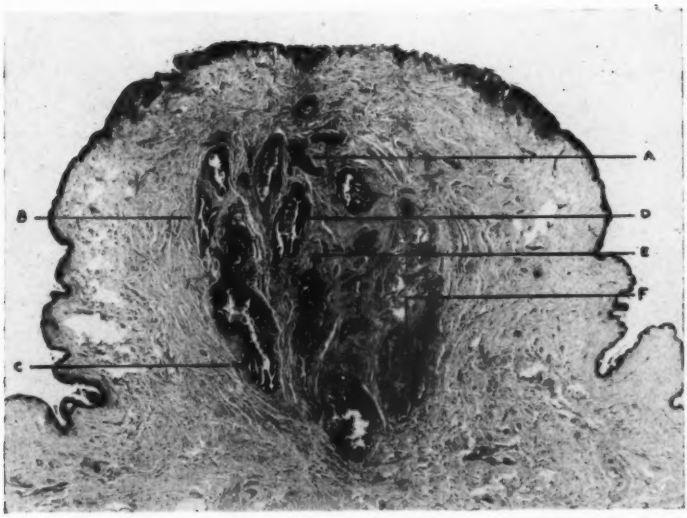

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Plate 4

Fig. 13 (case 1). Portion of duct A in Figure 12 is here shown more highly magnified. (This photomicrograph is not taken from the same section as Figure 12 but from a section near it in the series.) × 160.

Fig. 14 (case 1). Duct B of Figure 12 is here shown more highly magnified. × 80.

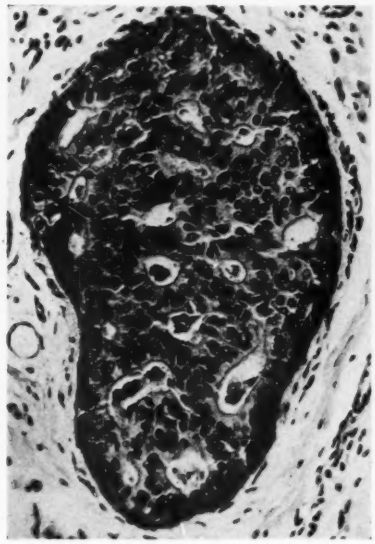
Fig. 15 (case 1). Duct C of Figure 12 is here shown more highly magnified. × 25.

Fig. 16 (case 1). Duct D of Figure 12 is here shown more highly magnified. × 55.

Fig. 17 (case 1). Duct E of Figure 12 is here shown more highly magnified. × 35.

Fig. 18 (case 1). Duct F of Figure 12 is here shown more highly magnified. × 45.

(Figs. 14 to 18 inclusive were all taken from the same section as Fig. 12.)



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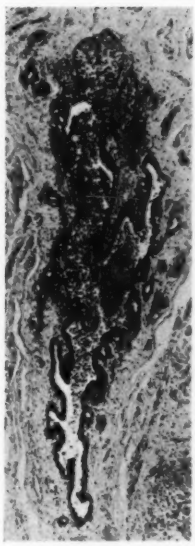

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Inglis

Paget's Disease of the Nipple

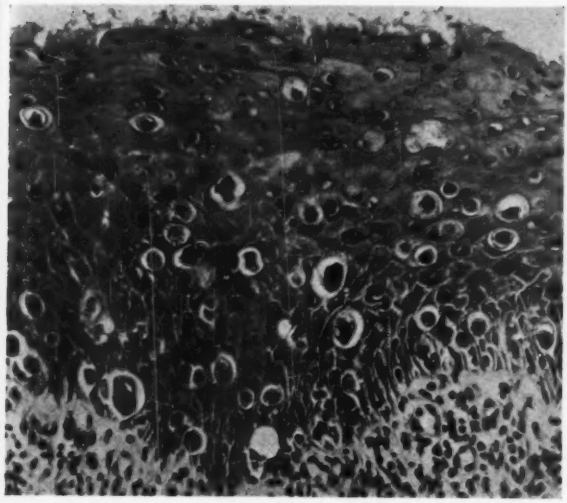
PLATE 5

FIG. 19 (case 2). The epidermal lesion. × 200.

FIG. 20 (case 2). Isolated cancer cell in interpapillary process. × 200.

FIG. 21 (case 2). Cancer spreading down wall of pilosebaceous follicle. × 50.

FIG. 22 (case 2). Portion of Figure 21 under higher magnification. A in Figure 21 corresponds with A in Figure 22. × 200.



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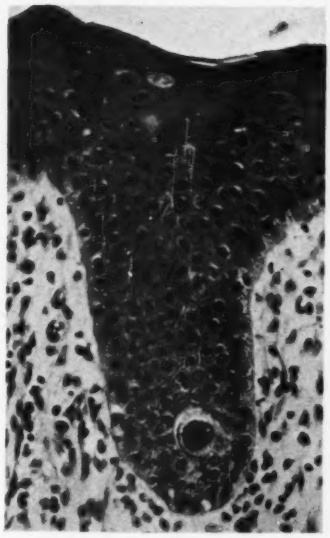

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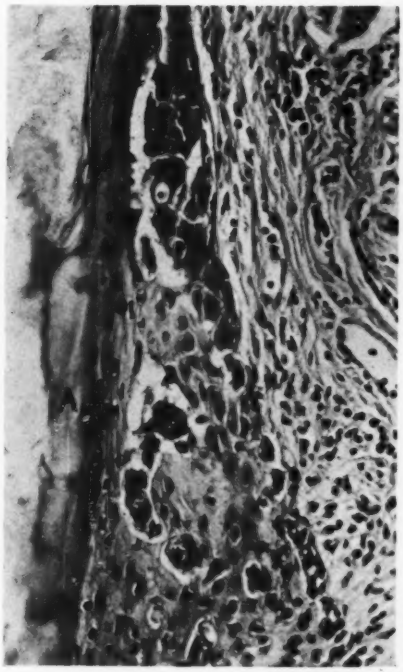
Inglis



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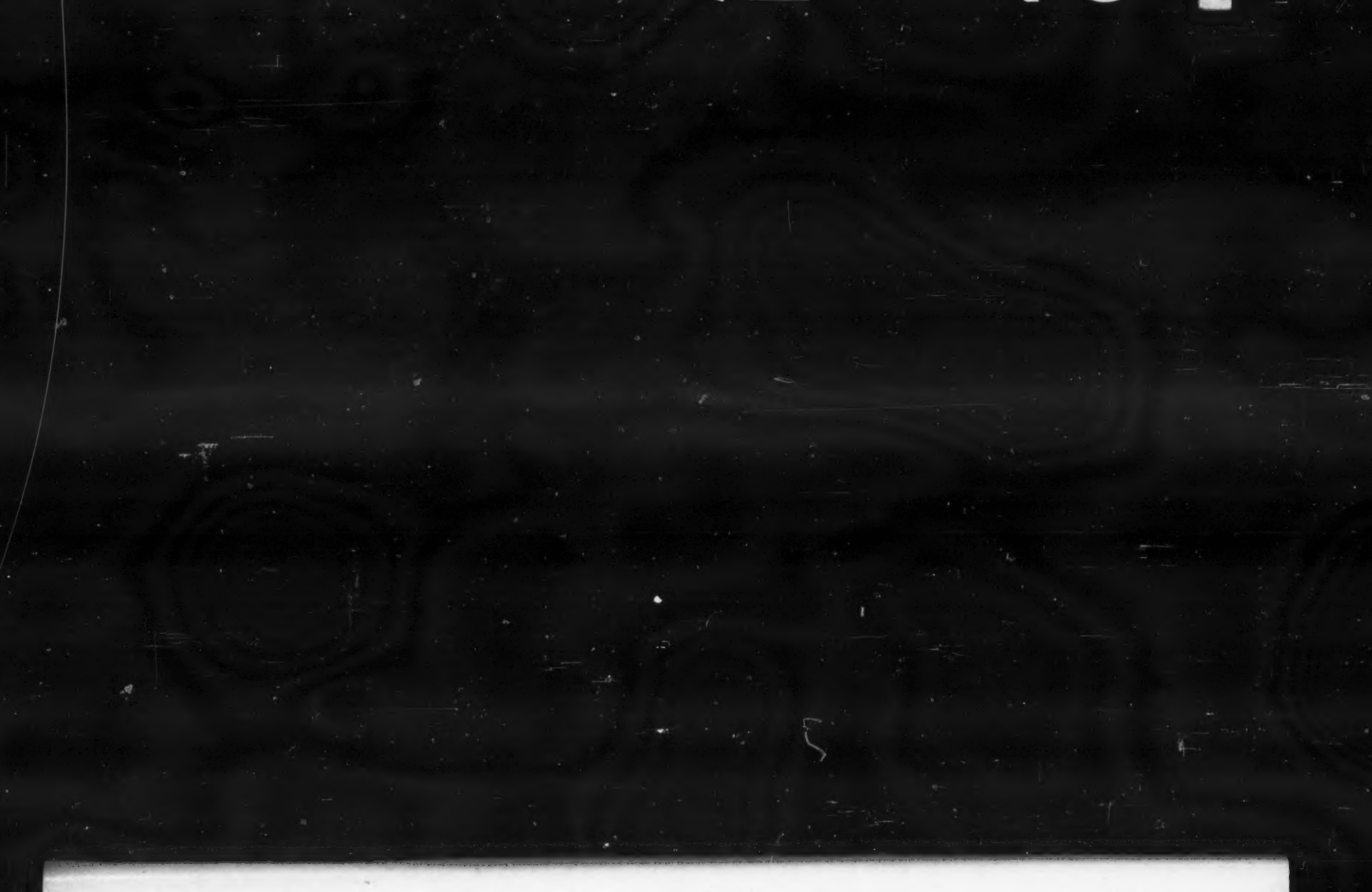
Paget's Disease of the Nipple

Plate 6

Figure 23 (case 2) and Figure 24 (case 2) are photomicrographs of the same duct. Figure 24 is not taken from the same section as Figure 23 but from one near it in the series. The lower limit of Figure 23 corresponds with the upper limit of Figure 24. Taken in conjunction, these two figures show the whole length of the intra-epithelial spread of cancer down the duct from the epidermis. In Figure 23 the cancer is continuous in the epidermis and in the duct. The lowest part of the duct in Figure 24 has not yet been reached by the downward-spreading cancer. Figure 23, × 100; Figure 24, × 100.

Figure 25 (case 2) shows intra-epidermal cancer which has spread a short distance down an accessory duct by the intra-epithelial route. × 100.

Figure 26 (case 2). A main lactiferous duct and adjacent portion of epidermis both showing extensive invasion by cancer. × 100.



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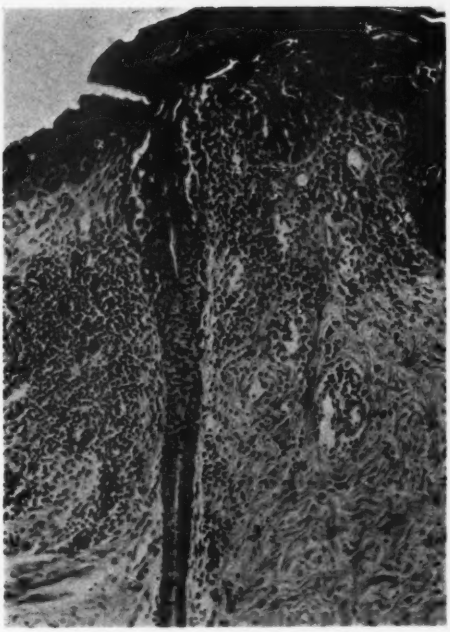

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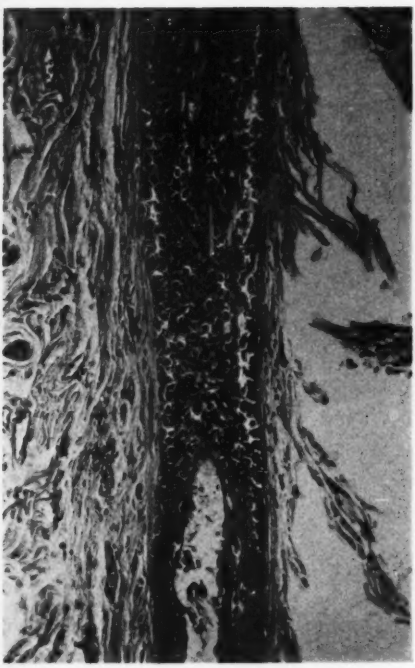

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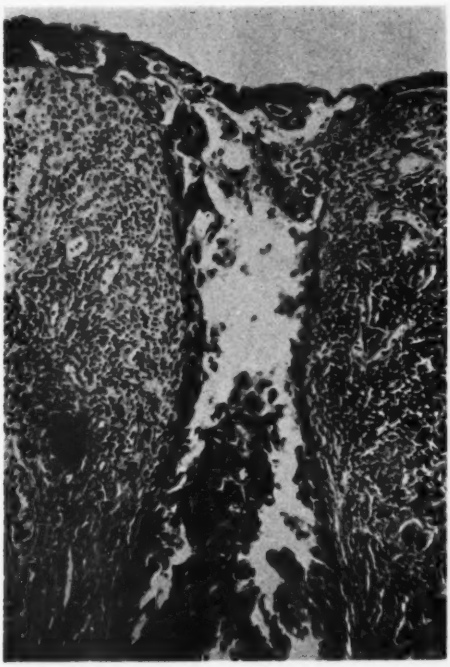

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Inglis

Paget's Disease of the Nipple

A CHRONIC GRANULOMATOUS DISEASE OF SWINE WITH STRIKING RESEMBLANCE TO HODGKIN'S DISEASE *

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It is apparent to anyone who reviews the many reports which relate to the nature and etiology of Hodgkin's disease that two serious obstacles block the way to a satisfactory solution of the problems involved. One is that it has not been possible to reproduce Hodgkin's disease in any of the lower animals, and the other is that thus far no one has observed a natural entity in any of the lower animals that critical workers will accept as identical with Hodgkin's disease.

There are several publications which deal with disorders in lower animals that bear certain superficial resemblances to Hodgkin's disease. Only a few of these need be cited since, in each, the difficulty encountered (readily appreciated by both author and reader) is essentially the same: all observations have been made on animals about which little or nothing was known previous to post-mortem examination, with the result that these studies could be made only on the basis of pathologic anatomy. A disease, or diseases, in the lower animals producing changes bearing some resemblance to the lesions of Hodgkin's disease has been described in dogs by MacMahon [1] and by Stalker, Schlotthauer, and Feldman; [2] in rabbits by Medlar and Sasano; [3] in swine by Hodgson; [4] and in the horse by Runnells and Benbrook.[5] Through the courtesy of several of these investigators we have had opportunities to study the materials forming the basis of their reports. In certain ways the diseases described present likenesses to Hodgkin's disease, but in none of the reported cases does the general picture more than suggest it. Even in those cases where the resemblance is most striking, the unavoidable incompleteness of the gross and histologic observations leaves the reader unconvinced.

The report which follows is subject to some of the criticism just directed toward earlier studies in this field; however, the cases are sufficiently numerous and the opportunity for gross and histologic study of the reactions involved has been comprehensive enough to make possible the recognition of a disease entity in the hog which if not actually Hodgkin's disease has a most impressive resemblance to it. To the best of our knowledge, this entity has not previously been described.

During the past 5 years one of us has been engaged in the study

* Received for publication, April 5, 1945.

of what had appeared to be a close relationship between infection by brucella and Hodgkin's disease. This investigation necessitated an extended study of experimental brucellosis in a variety of animals, including swine.[6] In the course of this work when it was necessary to make collateral studies on natural brucellosis in this animal, the Federal Bureau of Animal Industry supplied most of the materials. One of us, while at the Pathological Laboratory of the United States Bureau of Animal Industry in Denver, Colorado, had collected materials from swine, some showing characteristic lesions of natural brucellosis and others, lesions suggestive of brucella infection. These materials came from a variety of sources; most of them were sent to the laboratory by veterinarians in the Federal Meat Inspection Service for investigation and diagnosis.

Whenever the circumstances permitted, the materials were studied both anatomically and bacteriologically. Preliminary studies showed that the material could be divided into two rather sharply defined groups: one composed of cases of brucellosis, in which the diagnosis had been established both by the anatomic and histologic character of the lesions and by bacteriologic and serologic methods; the other presenting lesions that were quite different from those found in the cases of brucellosis, none of which yielded organisms of any sort when cultured. Only one case belonging to the second group showed a significant agglutination titer for brucella. While there were these highly important differences between the cases of the two groups, the gross alterations of the tissues, particularly the lesions in the spleen, were sufficiently alike to suggest a possible relationship. In the routine histopathologic studies of the materials of the second group, a resemblance of the lesions to those of Hodgkin's disease was noted, and the cases were classified as "Hodgkin's-like" granuloma. In view of the fact that these lesions also bore a certain resemblance to the lesions in hogs, either suspected or known to be infected by brucella, it was considered possible that they might represent certain phases in the development of the lesions produced by brucella in the hog. In our concurrent studies of experimental and natural brucellosis, we have been able to show that brucellosis in the hog is unrelated to the Hodgkin's-like granulomatous process,[6] but we were unable to determine the cause of the granulomatous lesion. Its resemblance to Hodgkin's disease was found to be so striking in further studies of this group that we felt that the cases should be recorded. The number of cases and the diversity of materials available for study make possible the recognition of a definite pathologic entity. Since these cases may prove useful in future comparative studies, the case studied most comprehensively and significant variations in other cases of this series will be fully described.

Report of Cases

Case 1

Hog ABF 2, an almost fully grown hog, suspected of having leukemia because of pronounced enlargement of the superficial lymph nodes, was sent for complete autopsy to Dr. S. S. Blackman of the Johns Hopkins Department of Pathology who was studying experimental leukemia. At autopsy a general enlargement of the lymph nodes due to peculiar fibrous changes was found. This was accompanied by considerable swelling and a profound, presumably granulomatous alteration of the structure of the spleen. Since microscopic examination showed no evidence of leukemia, all of the histologic preparations were given to us to be used in our studies of the Hodgkin's-brucellosis problem.

Many sections from each of the organs in this animal were studied microscopically by a variety of staining technics. With the exception of the presence of Sarcosporidia in the fibers of the voluntary muscles and of the heart, the lesions in this case were confined to the lymph nodes, the spleen, the bone marrow, and the liver. A description of each of these organs follows.

Lymph Nodes. The normal architecture persisted in some of the nodes, but in others it was greatly altered. In practically all nodes small areas showed relatively normal structural relationships. The earliest phase of the development of the lesion was characterized by a pronounced infiltration of the peripheral sinuses by cells of many types. In this infiltrate there were recognized a great number of eosinophilic leukocytes, a still greater number of reticulo-endothelial cells, many of which clearly were phagocytes, relatively few small round cells resembling lymphocytes and plasma cells, and a good many unidentifiable mononuclear wanderers. This reaction was followed by the development of a pronounced reticulo-endothelial proliferation which in some places converted the lymph node into a dense connective-tissue-like reticular scar. In this scar large mononuclear cells in considerable variety and eosinophilic polymorphonuclear leukocytes were numerous. Areas of focal necrosis, sometimes accompanied by hemorrhage, were conspicuous. Giant cells with multiple nuclei, seen here and there, usually took the form of large multinucleated macrophage-like structures, but occasionally resembled Langhans' giant cells. There was nothing in any of the lymph nodes that resembled a genuine epithelioid reaction nor a tubercle. Giant cells of the Dorothy Reed type were not present. The lesion, thus, was distinctly granulomatous; it resembled rather strikingly the very dense sclerosing lesion that characterizes the far advanced stage of Hodgkin's disease. Virtually all of the lymph

nodes studied histologically showed this type of advanced sclerosing reaction (Figs. 1 and 30).

Spleen. In the spleen the lesions occurred regularly and most characteristically in relation to the malpighian bodies. The normal cells of these structures gradually gave place to cells of a totally different kind, which bore a striking resemblance to the mononuclear cells that characterize the Hodgkin's lesion. As this proliferative reaction developed, a great quantity of hyaline reticulum was laid down. Eventually the cellular components of the lesion disappeared, leaving the malpighian body converted into a hyaline scar. Grossly, this reaction appeared as tiny gray nodules throughout the splenic pulp. Another characteristic lesion, somewhat different in its development but similar in its outcome, consisted of marked proliferation of the reticulo-endothelium, forming relatively large nodules of a highly cellular character. The proliferating cells maintained a degree of morphologic uniformity which in places suggested genuine neoplasia, but the nodules thus produced, in time were converted into a dense reticular scar similar to that described in the malpighian body. Although the reticulo-endothelial hyperplastic reaction is important, equal emphasis should be placed upon the presence of great numbers of eosinophilic leukocytes, hemorrhage, and giant cells of several varieties. As the lesion developed it sometimes obstructed the arterioles to produce relatively large infarct-like areas. The coalescence of these lesions produced an extensive nodular induration of the greatly enlarged spleen. A quantity of iron-containing pigment was scattered throughout the much altered splenic substance. The general transformation of the spleen by this granulomatous process was indistinguishable from that of Hodgkin's disease of the advanced sclerosing type; it bore no resemblance whatever to other lesions in the human spleen with which we are familiar. The cell complex that made up this granulomatous reaction was that of Hodgkin's disease, and the different types of giant cells that accompanied the mononuclear and polynucleated cells can be duplicated in sections from lesions of comparable age and development in the Hodgkin's process. Were this lesion found in the human spleen, the case would be readily accepted as one of Hodgkin's disease (Figs. 3, 13, 14, 15, and 16).

Bone Marrow. The bone marrow from one of the long bones and from the body of one of the vertebrae was studied. In both locations the lesions were focal. The cytologic composition of the lesion in the bone marrow was in every way comparable to that in the spleen. The lesion was distinctly a granuloma, characterized by a mixture of mononuclear cells, chiefly plasma cells and what appeared to be lymphocytes, leukocytes, and giant cells filling the meshes of a hyaline reticu-

lar scar. Some of the lesions were older than others, the tissues being almost nothing but scar. The uninvolved bone marrow had a normal cellular composition (Figs. 6, 28, and 29).

Liver. There was a diffuse round cell infiltration throughout the portal fibrous tissue of the liver. In some places there was considerable extension of this infiltration into the neighboring lobules, resulting in destruction of many of the liver cells. In affected areas a rich reticulum developed, thereby increasing the thickness and the density of the fibrous tissue capsule that normally surrounds the hepatic lobules. In addition to these periportal granulomatous infiltrations a similar reaction sometimes occurred within the lobule, presumably independent of the portal reaction. These intralobular lesions gave rise to extensive destruction of the hepatic parenchyma and its replacement by a granulomatous reaction. The cells which made up the reaction within the lobule and also in the portal areas were predominantly small, round elements, mixed with many eosinophilic leukocytes and a variety of mononuclear cells not easy to identify. It was obvious from the character and distribution of the lesions that long-continued progress of the disease followed by healing of the lesions eventually would result in an extensive cirrhosis of the liver. The obvious pathogenesis of the actively progressing but early lesions in this case makes it easy to understand the regular occurrence of cirrhosis of the liver in the other cases of this series. In the hepatic granulomatous reaction there was little production of giant cells. In all other respects the hepatic lesion was essentially the same as that in the spleen, the lymph nodes, and the bone marrow (Figs. 7, 8, 9, and 11).

In none of the lesions was it possible to demonstrate organisms of any variety either by culture of the tissues or by special staining of the histologic preparations.

In view of the clinical suspicion that this animal was suffering from leukemia, it should be emphasized that studies of blood clots in sections revealed no evidence of an alteration in the cytologic composition of the circulating blood. In the sections, furthermore, nothing was found to indicate a leukemic process.

Case 2

Hog ABF 9, D-344, was approximately 1 year old. At post-mortem examination, Dr. H. L. Shorten of the Federal Meat Inspection Service, Denver, Colorado, noted multiple tumor-like nodules, approximately 5 to 8 mm. in diameter, in the spleen, the liver, and the kidneys. The liver showed pronounced cirrhosis. The nodules in the spleen and the liver were white and fibrous; those in the kidney were similar but perhaps somewhat softer. No data relating to the condition of the

other organs were available. The lymph nodes were not mentioned in the very brief protocol. No material was available for studies of agglutination titers or for bacteriologic examination. Our observations of the small pieces of tissue from spleen, liver, and kidneys added nothing to the original descriptions made by Dr. Shorten, but further information was gained from histologic studies.

Spleen. The lesions in the spleen in this case were identical with those in case 1, which appeared to have developed first in the malpighian bodies. The youngest lesions were characterized chiefly by proliferation of large mononuclear cells. The subsequent development of this reaction was featured by a certain amount of necrosis and marked production of hyaline reticulum; the end-result was the transformation of the malpighian body into a rather dense reticular scar. Accompanying the proliferation of the large reticulo-endothelial cells was an accumulation of eosinophilic leukocytes and a variety of small round cells. Hemorrhage was conspicuous. The larger nodules seen grossly in the spleen were the result of proliferation of the reticulo-endothelial cells accompanied by the formation of many multinucleated giant cells. The development of the reticular ground substance in these nodules was impressive. As in case 1, the lesions appeared much like tumors. Nodules of this type eventually lost their cellular character and in the end consisted of rather dense reticular scars in which a few eosinophilic leukocytes, multinucleated giant cells, and a variety of small mononuclear cells persisted. The unaltered splenic substance lying between the lesions showed considerable congestion, probably from portal obstruction, the result of accompanying cirrhosis of the liver (Figs. 4, 12, 17, and 20). (See also Figs. 18 and 19.)

Liver. The liver had dense periportal scarring throughout, which divided it into many small compartments, each containing a little mass of liver cells which belonged to the original lobule. The great bands of scar tissue that surrounded these lobules contained the bile ducts and showed a marked inflammatory reaction of a granulomatous character. The composition of the exudate here was like that in the splenic lesions. In the active granulomatous reaction, giant cells were inconspicuous just as they were in case 1. The more outstanding features were the great number of eosinophils and the extensive production of scar tissue (Fig. 10). (See also Figs. 2, 7, and 11.)

Kidney. Small nodular lesions, exactly like those in the spleen in so far as cellular composition is concerned, were present in the kidney. Giant cells, however, were not conspicuous; the eosinophilic reaction, on the other hand, was conspicuous.

In none of the sections were there bacteria or parasites of any sort.

The lesions in the spleen were identical with those in case 1, and if seen in the human spleen would, without question, be diagnosed Hodgkin's disease of advanced type. The lesions in the liver were like those in case 1, with the exception that the process had advanced much further, resulting in greater destruction of the hepatic tissue and more pronounced periportal scarring.

Case 3

Hog ABF 10, D-548, was 1 year old. Autopsy was performed by Dr. I. L. Barstow of the Federal Meat Inspection Service, Denver, Colorado. The liver was diffusely scarred, enlarged, and indurated. Similar changes were noted in the kidneys, and tubercle-like foci were scattered throughout the spleen. No other gross abnormalities were noted. The gross appearance of the spleen, as we saw it, was not unlike that in cases 1 and 2, with the exception that the lesions in this case seemed to be much older because of their indurated, fibrotic character. The many small nodules scattered irregularly throughout the splenic tissue were usually discrete and well circumscribed, but not definitely encapsulated. Here and there they tended to coalesce. In gross appearance the lesions were like those of tuberculosis or brucellosis, and our first impression was that they were due to brucella infection. A considerable degree of what appeared to be scarring was obvious in the tissue from the kidneys. The appearance of the liver was that of diffuse nodular cirrhosis. In the more fibrous areas between the nodules of hepatic cells little yellow foci were found, suggesting either necrosis of liver cells or a granulomatous inflammatory reaction. A description of the histologic appearances of the spleen, liver, and kidney follows.

Spleen. Scattered throughout the sections of the spleen were small, rather sharply defined nodules which consisted chiefly of scar tissue containing clumps of eosinophilic leukocytes. The outer margin of each of the nodules showed extensive infiltration by eosinophilic leukocytes and small mononuclear wandering cells. The lesion was not encapsulated by fibrous tissue, but condensation of the normal splenic tissue toward its outer margin clearly defined its limits. There were no giant cells. Here and there were spindle-shaped spaces like those sometimes seen when crystals are dissolved from the tissues. There was a considerable amount of iron-containing pigment in the lesion. There was virtually no lymphoid tissue present in the spleen. The malpighian bodies were almost devoid of cells, which had been replaced by scar tissue.

It is evident that the typical splenic lesion in this case differs con-

siderably from that found in cases 1 and 2. Our interpretation of the lesions in this case is that they represent a granulomatous process in an advanced stage of healing. Although the lesions are now thought to represent the complete healing of a process identical with that seen in cases 1 and 2, they were considered originally to be the completely healed lesions of brucellosis, like those we have described in a previous communication [6] (Figs. 18 and 19).

Liver. The sections from the liver showed extensive periportal scarring. In the thick, dense bands of fibrous tissue surrounding the little nodules of persistent hepatic tissue there were collections of eosinophilic leukocytes and a variety of wandering cells. The lesion, like that in case 2 (Fig. 10), is interpreted as a granulomatous periportal hepatitis which has undergone almost complete healing.

Kidney. No active lesions were found in the kidney, but there was extensive interstitial scarring; in some areas this was focal, in others diffuse. Nothing was seen to indicate the nature of the process responsible for destruction in the kidney.

Case 4

The post-mortem examination of hog ABF 38, D-675, was also performed by Dr. I. L. Barstow. He observed that the spleen was enlarged to several times its normal size, with suet-like foci distributed throughout. The liver also was enlarged and was thought to show some evidence of degeneration. The submaxillary, portal, and renal lymph nodes appeared hyperplastic. Material consisting of lymph nodes, spleen, liver, and bone marrow was made available to us for study, in which we were able to confirm Dr. Barstow's observations. The lymph nodes we found to be firm in consistency, mottled, and rusty brown. They were not unlike those seen in advanced Hodgkin's disease. The lobular structure of the liver was prominent, suggesting definite cirrhosis. The spleen was remarkable in appearance with scattered little gray foci, elastic in consistency, which were rather sharply defined but not encapsulated. They varied greatly in size, some being just barely visible, others reaching a diameter of from 2 to 3 mm. In some places the lesions coalesced and produced a complete distortion of the splenic structure. Although there was some necrosis, the greater alteration of the tissue appeared to be the result of replacement of the normal splenic substance by the proliferation of a new kind of tissue. Pigmentation by iron was so pronounced that in some places the spleen had a definite porphyry-like appearance. The alterations in the splenic substance were indistinguishable from those regularly observed in

Hodgkin's disease. The gross lesions in the lymph nodes, liver, and spleen are shown in Figure 2. A histologic description of the lesions follows.

Lymph Nodes. The general architecture of the lymph nodes did not appear significantly disturbed, but there were areas in which a granulomatous inflammatory reaction caused considerable change. These areas were most numerous at the periphery of the node and along the course of the lymphatic sinusoids that lead toward the medulla of the gland. The reaction was similar to that in case 1, except that, as yet, little reticulo-endothelial scarring had occurred, indicating that the reaction in the lymph node was of a much earlier type. Another feature whereby it differed was the presence of many giant cells which were predominantly of two varieties. One was a multinucleated structure that was in every way like the Dorothy Reed cell of Hodgkin's disease. The other was a large, usually multinucleated but sometimes mononucleated cell with an abundance of cytoplasm which was dense and which stained rather intensely with eosin. In some instances the single nucleus in this giant cell was drawn out into a long cylindrical mass, and sometimes it appeared as a single central mass with several lobulations of irregular outline. Giant cells of the latter type were like those seen sometimes in the splenic pulp and always in the bone marrow. These megakaryocyte-like giant cells appeared in abundance also in the spleen. Another feature in the lymph nodes which was more conspicuous in this case than in case 1 was the presence of relatively large foci of proliferating mononuclear reticulo-endothelial cells. Eosinophils and other mononuclear cells were found, but not in sufficient numbers to suggest a neoplastic reaction. These little foci were like those that appeared in the spleens of cases 1 and 2. (They also formed a conspicuous proportion of the lesions in the spleen in this case.) Altogether, the lesion in the lymph node was a granuloma which, if seen in a human lymph node, would surely have been considered entirely compatible with human Hodgkin's disease (Figs. 31 and 33).

Spleen. The reactions in the spleen in this case were identical with those in case 2, being most conspicuous in connection with the malpighian bodies. The cytologic composition of the lesion was that of a granuloma. Eosinophils were prominent, and large mononuclear cells of varied structure, scattered among the fibrils of a rather dense reticulum, were characteristic. Among the mononuclear cells were many giant cells, some of them like the Dorothy Reed cells of Hodgkin's disease, others like the megakaryocytes of the bone marrow, and still others like large multinucleated fibroblasts. A striking feature of

the nucleus of the multinucleated giant cell was the sharply defined, slightly eosinophilic nucleolus. Similar nucleoli were commonly found in the mononuclear cells of the large variety which represented a cytologic type basically the same as that of many of the giant cells. Between the lesions, which were somewhat focal in distribution, the splenic pulp was sparse, the sinusoids were packed with blood, and there was some increase in fibrous tissue. Intense pigmentation of the scarred areas by deposits of iron was characteristic. The lesions, which obviously developed as small foci, tended to coalesce, leaving a little intervening, normal, splenic substance. Some of the sharply outlined nodules were composed of proliferating reticulo-endothelial cells with a pronounced reticulum; they resembled in every respect the lesions in the lymph nodes and suggested a genuine neoplastic development. An interesting and important feature of the small tumor-like areas of reticulo-endothelial proliferation was the presence of multinucleated giant cells like those already noted in other lesions of the spleen. It is clear that the alteration of the splenic substance in this case conforms to the histologic pattern which is characteristic of the lesions in all cases included in this report. The reaction here, however, appears more active than in any of the other cases except, perhaps, case 2. The reaction in the spleen is indistinguishable from that of Hodgkin's disease (Figs. 22 to 27).

Liver. The sections of the liver showed a considerable periportal granulomatous inflammation in which eosinophilic leukocytes were prominent; the other cells were chiefly small mononuclear elements. There were no giant cells, and very little reticulo-endothelial scarring. The periportal markings of the liver lobules were conspicuous, but there was not sufficient alteration of the hepatic structure to justify one in describing this liver as cirrhotic, although in the gross this organ did seem to be distinctly nodular.

Bone Marrow. Nothing unusual was seen in the bone marrow which was composed entirely of fat.

The blood serum of the animal from which these tissues came was positive for brucella agglutinins in a dilution of 1:50. A diphtheroid organism was recovered in culture from the spleen. Guinea-pig inoculations of the tissues produced no disease.

Case 5

The materials available for our study of hog ABF 52, 14,300, were limited to small sections of spleen, liver, and one portal lymph node. Leukemia was suspected by Dr. G. A. Franz of Indianapolis, who did the autopsy, and the animal was sent to the Denver laboratory for diagnosis. The liver was found to be greatly enlarged, extensively

scarred, and finely nodular. The gross picture of the greatly enlarged spleen was identical with that of case 4. The lymph nodes from the hilum of the liver were enlarged and contained scattered, gray, poorly defined areas which tended to extend from the capsule toward the medulla. All tissues of this animal were slightly jaundiced. No bacteriologic studies were made. The changes in these tissues as seen microscopically may be described as follows.

Spleen. The lesion in the spleen conformed exactly to the pattern described in detail for case 4.

Lymph Node. The lesion in the lymph node was like that seen in the nodes of case 4 with the important exception that giant cells were more numerous and tended to conform to the pattern of the Dorothy Reed cell. Another point of difference is worth noting. The rather pronounced reticulo-endothelial reaction was pseudo-epithelioid in character in this node and, therefore, the lesion was composed of tissue of a somewhat homogeneous type. This was most noticeable at the periphery of the node where the lesion involved the peripheral sinuses, but it extended also along the lymphatic channels toward the medulla. Eosinophils were numerous. The architecture of the node was not greatly altered except in those areas where the lesions were best developed (Fig. 32).

Liver. There was extensive periportal scarring in the liver, but a receding granulomatous reaction was still active. Eosinophils were numerous. Mononuclear cells of considerable variety made up the rest of the exudate. Evident healing of the reaction had given rise to thick bands of fibrous tissue which surrounded masses of cells that represented original lobules. The histologic picture was that of extreme portal (biliary) cirrhosis.

Case 6

Hog ABF 55, D-910, was 18 months old. At the autopsy, performed by Dr. O. E. Jung, Sr., of the Federal Meat Inspection Service, St. Louis, Missouri, the spleen was found to be greatly enlarged, perhaps eight times normal size. It was hemorrhagic, with numerous, small, suet-like foci distributed throughout. The liver was somewhat enlarged and showed a pronounced degree of cirrhosis. There was also enlargement of the lymph nodes. Neither bacteriologic nor serologic studies were possible nor was the material suitable for animal inoculations. Histologic sections of the materials available revealed the following changes.

Spleen. There was extensive scarring of the spleen, clearly the result of the coalescence of multiple lesions. These lesions appeared active only here and there but, wherever they were found to be progressing,

they were identical with the focal lesions described in cases 2 and 4. Since the general histologic picture conformed exactly to the pattern described in detail in these cases, further description of the lesions is unnecessary. It is obvious that the changes in the spleen are the result of a granulomatous process in an advanced stage of healing (Fig. 5).

Liver. Sections showed extensive periportal scarring of the liver, apparently the result of a granulomatous reaction which still could be seen here and there in the scar tissue. Many liver cells, particularly at the periphery of the lobules, had been destroyed. Although the process was still active, it was obvious that it was rapidly regressing.

Lymph Node. No histologic preparations of the lymphoid tissues were available.

Case 7

The post-mortem examination of hog ABF 56, D-625, was performed by Dr. A. J. Clark, of the Federal Meat Inspection Service, Fort Worth, Texas. He found the spleen to be approximately 30 inches in length and correspondingly enlarged in width and thickness. The liver was enlarged and cirrhotic. The visceral lymph nodes were swollen and soft. There were no gross alterations of kidneys and lungs. The microscopic appearances of the tissues may be noted briefly.

Liver. There was a pronounced cirrhosis of the liver, the dense scarring being situated chiefly about the bile ducts in the portal areas. There was only slight activity of the destructive process, which appeared granulomatous as in other cases in this series.

Spleen. The picture in the spleen conformed in general to the pattern of the splenic alterations in the other cases of this group except that the original focal scarring had now become diffuse as a result of coalescence of the lesions. There was still some activity of the granulomatous process that preceded the scarring. This case is, in general, like case 5, in which the granulomatous inflammation had almost come to an end.

Lymph Node. The architecture of the node was not generally altered. There were foci of granulomatous reaction here and there along the course of the lymphatics extending from the capsule toward the medulla. The cytologic composition of this reaction was like that described for cases 4 and 5.

Comment

When the pathologic anatomical data are examined critically, it becomes obvious that these cases present a clearly defined entity. Case 1 demonstrates that all of the tissues constituting the reticulo-endothelial

system are involved in the basic pathologic process. A consideration of the seven cases in the series leads us to the conclusion that one basic pathologic process, a granulomatous inflammation, is responsible for the lesions in every case. Focal and sometimes tumor-like proliferation of the reticulo-endothelial cells in some of the lesions in both spleen and lymph nodes need not lead to confusion since such pseudoneoplastic processes are commonly found in many granulomatous reactions of proved causation.

The limitations placed upon the study of these cases by the circumstances under which the materials were obtained have made it impossible for us to obtain positive information about the cause. The lesions characteristic of these cases, when seen by veterinarians conducting meat inspection, are usually considered to be either granulomatous inflammation or neoplasia. Of our seven cases, three (cases 4, 6, and 7) were diagnosed clinically as lymphocytoma; two (cases 1 and 5), as leukemia; one (case 2), as sarcoma; and one (case 3), as tuberculosis. From our studies of natural and experimental brucellosis in the hog, it is possible to say that there is no indication that brucella has an etiologic relation to this granuloma nor is there any justification for considering any of the animal parasites or any form of *Mycobacterium tuberculosis* as a possible etiologic agent. In none of the lesions were we able to demonstrate organisms of any sort even though this series of seven cases represents all stages in the development of the granulomatous reaction which characterizes the entity. A careful study of the genesis of the lesions of all types in these cases leaves little support for the suggestion that they are neoplastic.

The almost constant occurrence of cirrhosis of the liver in these cases is worthy of comment. Primary destruction of the hepatic parenchyma followed by extensive scar formation and portal venous obstruction might easily give rise to enlargement and extensive scarring of the spleen. The type of reaction seen in the portal areas of the liver in most of this group might possibly follow chronic progressive hepatic destruction by a variety of agents. Thus it would be unnecessary to assume, as we have, that the hepatic granulomatous reaction and the lesions of the spleen are of identical, primary, specific causation; it is conceivable that the splenic lesion is simply a consequence of the hepatic portal scarring. However, even in this short series of cases, cirrhosis of the liver of such severity as to result in portal obstruction and scarring of the spleen was not always present. In fact, fibrosis and granulomatous reactions in the spleen (also in the lymph nodes) appear in some cases in which there is almost complete absence of

hepatic alteration; furthermore, the splenic lesion clearly is not a simple fibrosis like that which results from chronic congestion. In view of these facts, it is impossible to interpret these cases as instances of primary cirrhosis of the liver with peculiar sequellae. Even were one inclined to do this, the demonstration of generalized reticulo-endothelial disease in case 1, which was comprehensively studied, would make such a position untenable.

The entity recognized in this group of cases bears an extraordinary resemblance to Hodgkin's disease, if it actually is not that disease. We have attempted to make the comparison as objective as possible by supplementing the descriptive details in the protocols with illustrations.

In conclusion, it should be noted that a careful comparison of the lesions found in this group of cases with a variety of tumors in the hog, particularly the genuine tumors of the reticulo-endothelial system, as well as the leukemias, has shown conclusively that the cases do not represent variants of these diseases. This distinction has been made without difficulty. The observations made by one of us in the routine inspection of swine on the killing floors of packing houses as well as in the laboratory indicate that the entity described in this paper, like Hodgkin's disease, is relatively rare.

Summary

1. Seven cases of a relatively rare chronic granulomatous disease of swine are described. This disease of unknown causation, apparently not hitherto recognized as an entity, is characterized by a striking pathologico-anatomic resemblance to Hodgkin's disease, if it does not actually represent that disease in the hog. Further studies of this disease designed to determine its cause are in progress.

2. The unlikelihood that this Hodgkin's-like granulomatous disease is related to tuberculosis, brucellosis, or to any of the other granulomatous disorders of known cause is emphasized.

We wish to acknowledge our indebtedness to Dr. S. S. Blackman of the Department of Pathology, Johns Hopkins Medical School; and to Dr. H. L. Shorten and Dr. I. L. Barstow of Denver, Colorado; Dr. A. J. Clark of Fort Worth, Texas; and Dr. G. A. Franz of Indianapolis, Indiana, all of the Federal Meat Inspection Service, through whose careful observations the materials forming the basis of this study were accumulated. The senior author is particularly indebted to Dr. Adolph Eichhorn, formerly Chief, Pathologic Division, United States Bureau of Animal Industry, and to Dr. John R. Mohler, formerly Chief, United States Bureau of Animal Industry, whose cooperation has made possible not only this but other correlated studies.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 7

FIG. 1. Case 1. An enlarged and extensively scarred cervical lymph node in full section (× 4). The structure of the node is extensively altered by a granulomatous reaction resulting in a dense reticular fibrosis. Near the center and at the right, irregular areas of normal lymphoid tissue persist.

FIG. 2. Case 4. In the upper half of this photograph are three lymph nodes (one unsectioned, two sectioned), approximately actual size, which show enlargement and disturbed architecture. Below these are two blocks of liver, nodular in character, and at the bottom four blocks of spleen containing gray, granulomatous lesions.

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2

PLATE 8

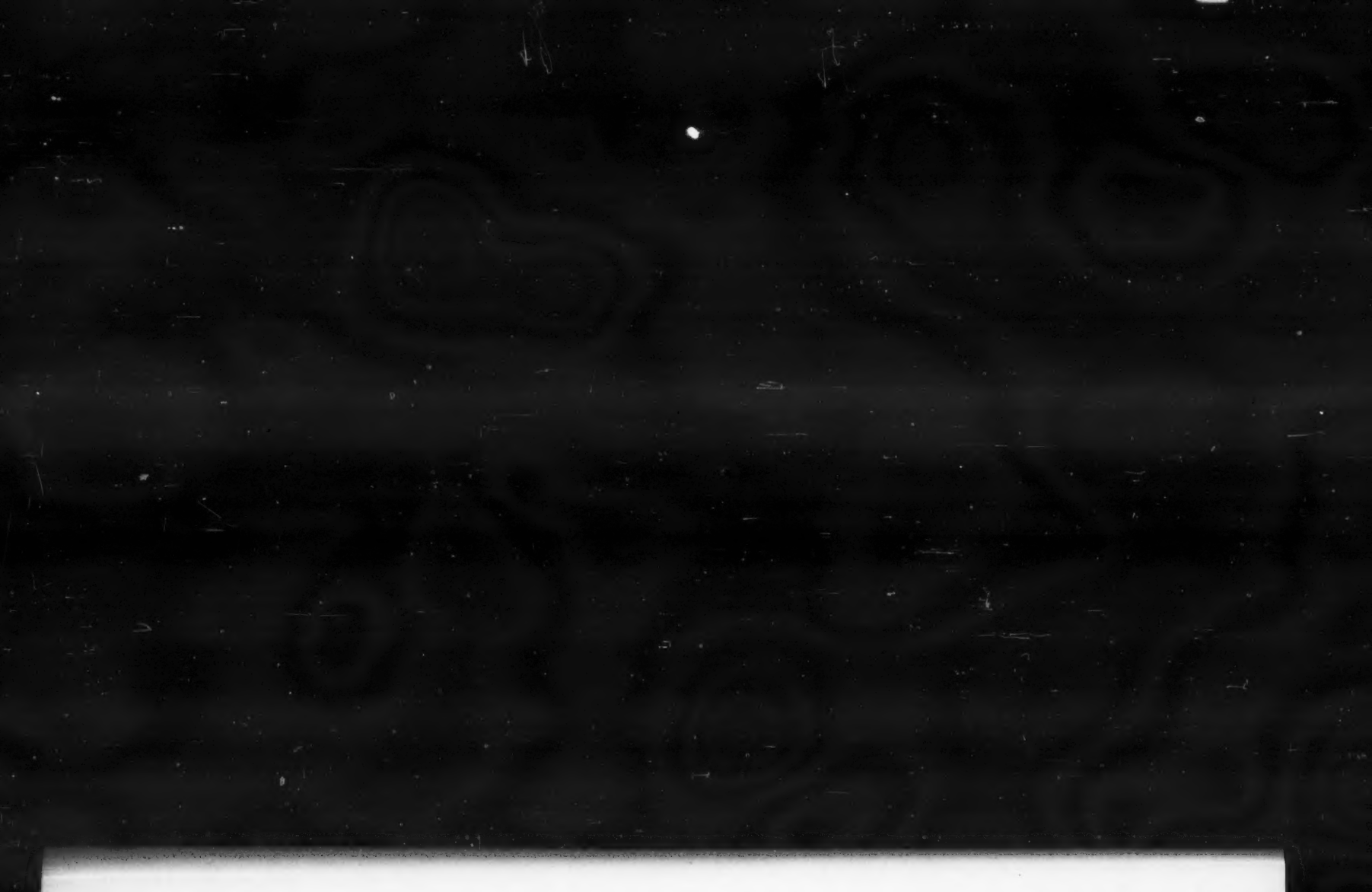
FIG. 3. Case 1. A section of the spleen (× 5) shows great alterations in structure produced by a widely distributed granulomatous reaction. The very small white dots are completely scarred malpighian bodies. The minute tumor-like focal reticulo-endothelial proliferations are similar to the large tumor-like nodules shown in Figure 4 from another case in this series. (See Figs. 13, 14, 15, and 16 for higher magnifications.)

FIG. 4. Case 2. A section of the spleen (× 5) shows focal tumor-like reticulo-endothelial proliferation and accompanying diffuse and focal granulomatous alteration of the splenic pulp. (See Figs. 12 and 17 respectively for higher magnification of the lesion in the malpighian body and the granulomatous reaction in the pulp.) At higher magnification the large tumor-like nodules present a picture identical with that of the small gray foci in case 1 (Figs. 3 and 16).

FIG. 5. Case 6. A section of the spleen (× 5) shows characteristic focal but coalescing granulomatous lesions. Figures 3, 4, and 5 together show the characteristic features of the spleen in this Hodgkin's-like granulomatous disease of swine.

FIG. 6. Case 1. A section of the bone marrow (× 5) shows granulomatous alteration and resulting scar. The lesion, essentially like those of the spleen and lymph nodes, is shown at higher magnification in Figures 28 and 29.

FIG. 7. Case 1. A section of the liver (× 5) shows a very early stage in the development of the periportal granulomatous inflammation. Higher magnifications are pictured in Figures 8, 9, and 11. In Figures 2 and 10 respectively are illustrated the gross and microscopic appearances of the far advanced and healing stages of this lesion in other cases of this series.



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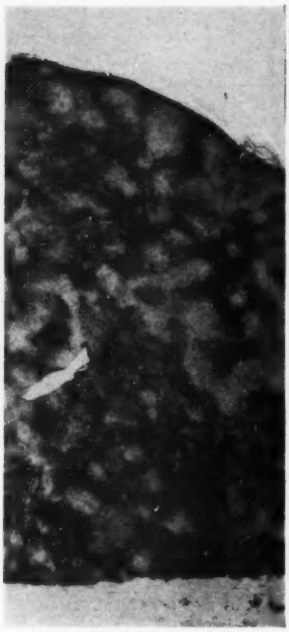

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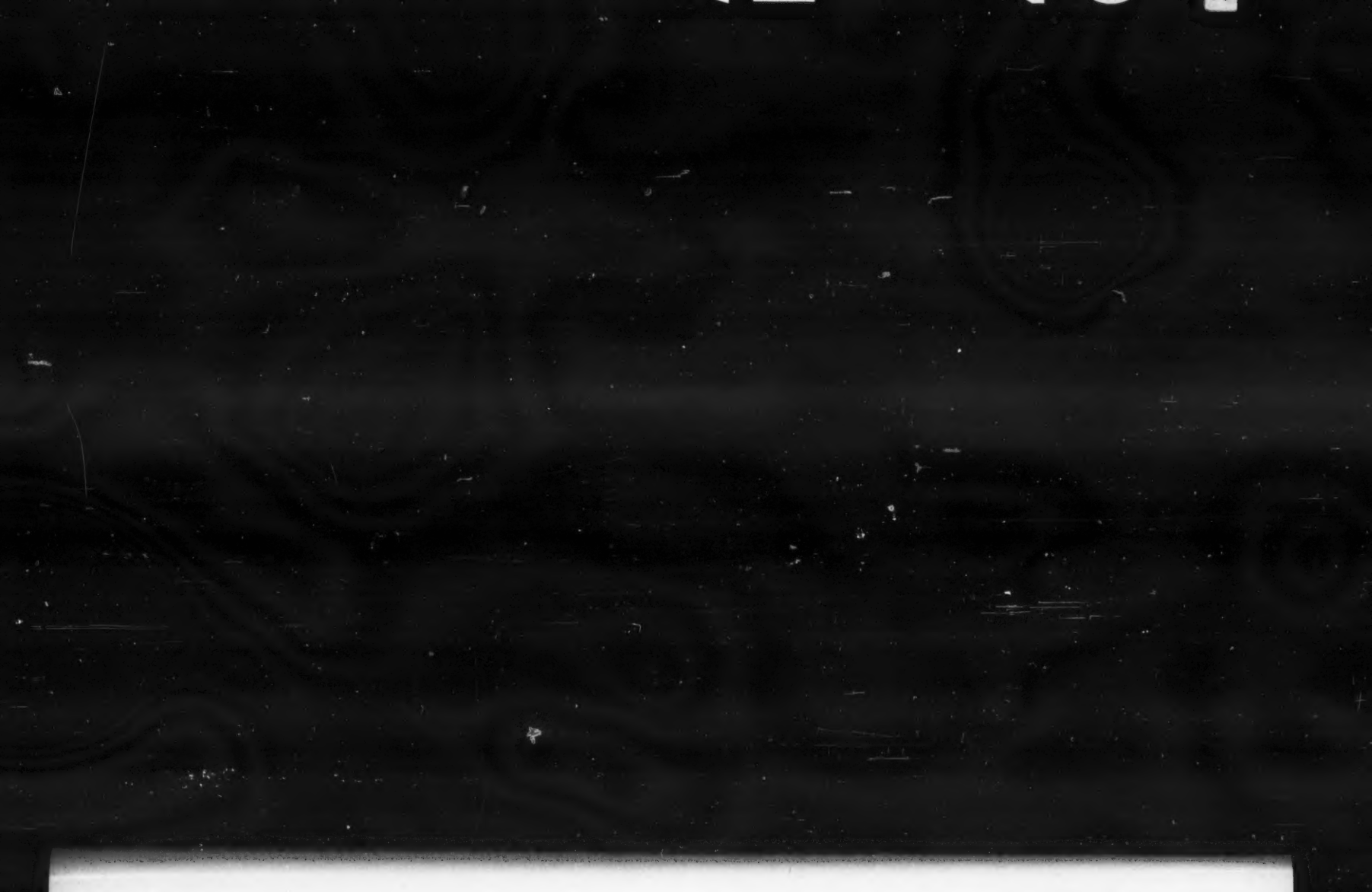
Forbus and Davis

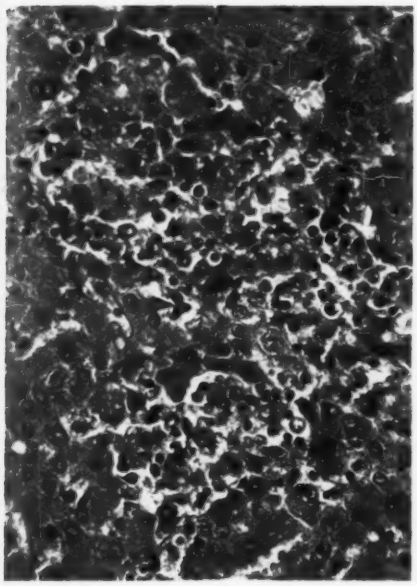
Disease of Swine Resembling Hodgkin's Disease

PLATE 9

FIGS. 8, 9, and 11. Case 1. Photomicrographs showing the typical early and progressing granulomatous reactions in the liver characteristic of the Hodgkin's-like disease of the hog. Figure 8 shows the lesion within the hepatic lobule (× 315). Figure 9 is the same at a higher magnification (× 485). Figure 11 is a typical periportal lesion (× 137). (See Fig. 7 for a low-power view of this liver, and Figs. 2 and 10 for the advanced stage in the development of the hepatic lesion as found in other cases of this series.)

FIG. 10. Case 2. A section of the liver (× 485) showing the advanced healing stage of the periportal granulomatous inflammation characteristic of the Hodgkin's-like entity. Of note are the dense scarring and the peripherally situated, persistent eosinophilic and mononuclear reaction. For comparison with Figures 8, 9, and 11. The gross appearance of this liver was that of typical nodular cirrhosis (Fig. 2).





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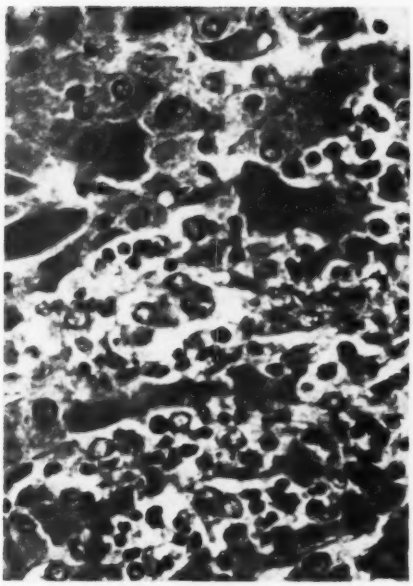

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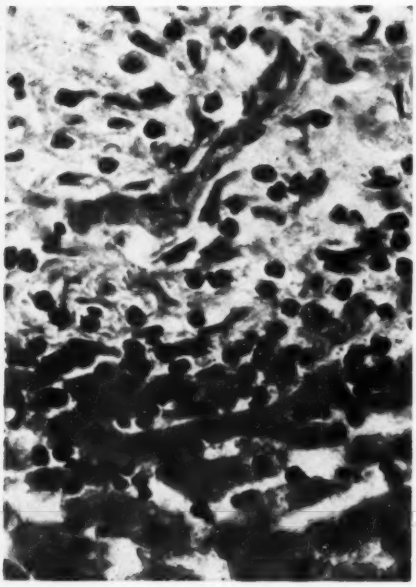

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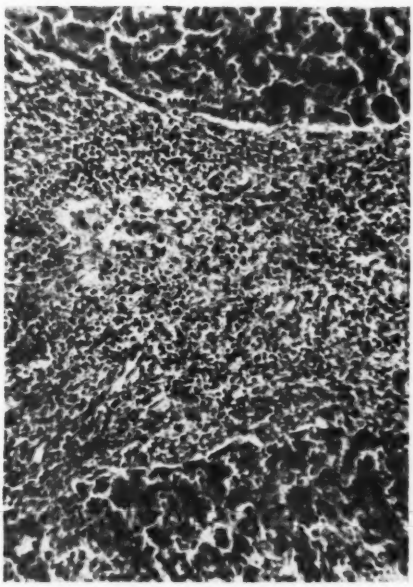

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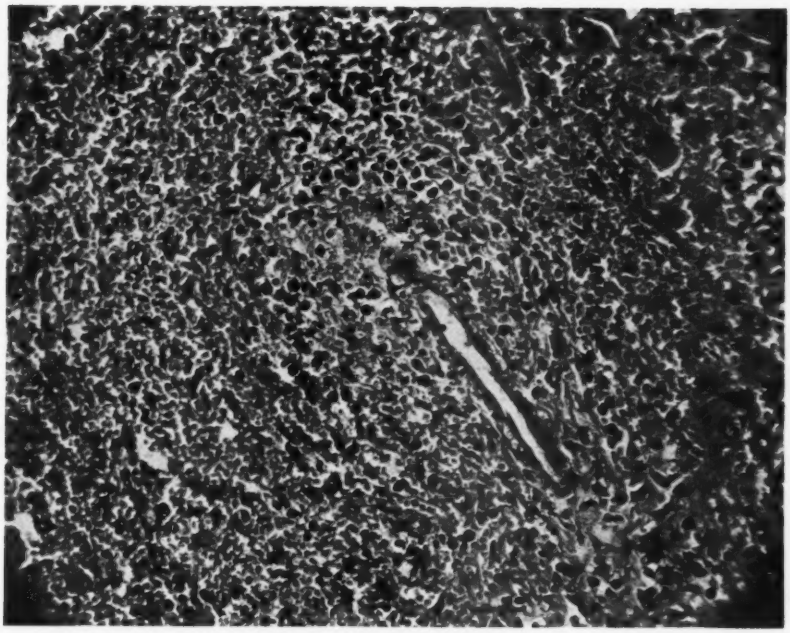
PLATE 10

FIGS. 12 and 13. Alterations in the malpighian bodies of the spleen typical of the Hodgkin's-like disease of the hog are seen in Figure 12 (× 285) from case 2 and Figure 13 (× 142) from case 1. These lesions are the result of near healing of a typical granulomatous reaction in the lymphoid tissue surrounding the splenic arteriole, and are histologically identical with the most typical and regularly occurring splenic lesion of Hodgkin's disease.



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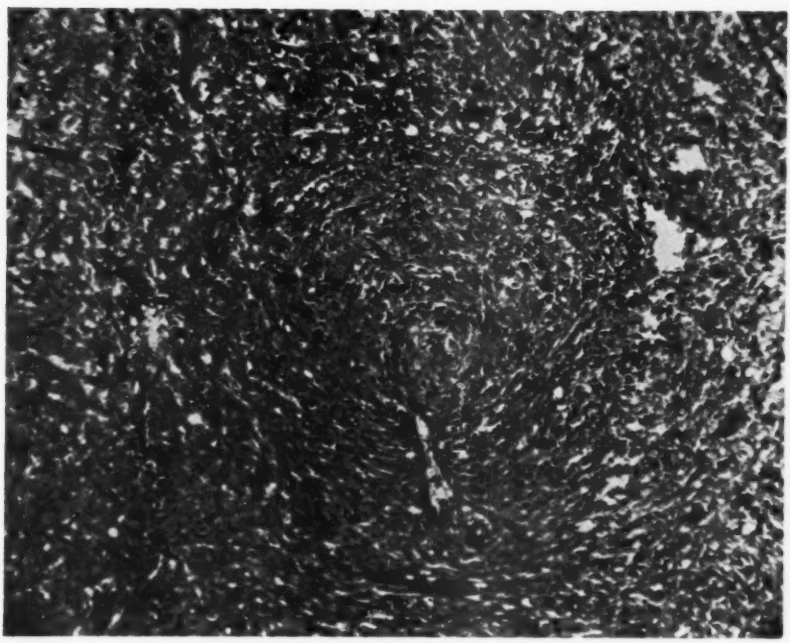

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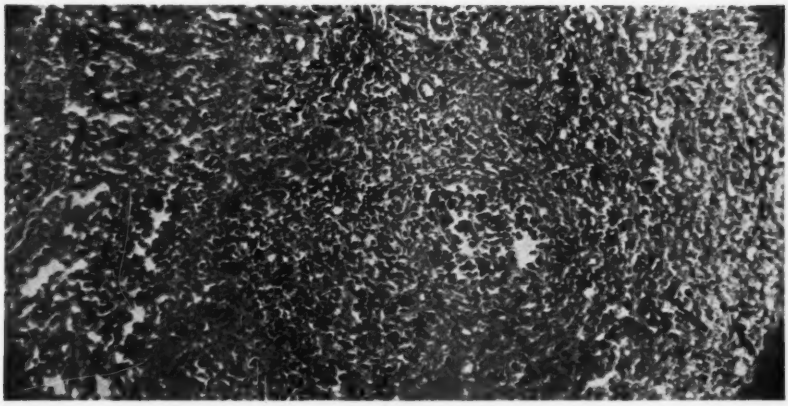
PLATE 11

FIGS. 14, 15, and 16. Case 1. Typical lesions in the spleen. Figure 14 (× 117) shows confluent focal granulomatous lesions with adjacent fibrosis. Figure 15 (× 345) shows a more diffuse type of granulomatous reaction in which eosinophils, large mononuclear, lymphoid, and multinucleated giant cells are conspicuous. Figure 16 (× 485) represents the typical, focal, tumor-like, reticulo-endothelial proliferation which gives rise to gross nodules in the spleen such as those pictured in Figure 4. (See Fig. 3 for a low-power photograph of this spleen.) Lesions of the type shown in Figure 16 eventually undergo extensive fibrosis, the result being a hard nodule microscopically like that shown in Figure 19. An intermediate stage is represented in Figure 18.



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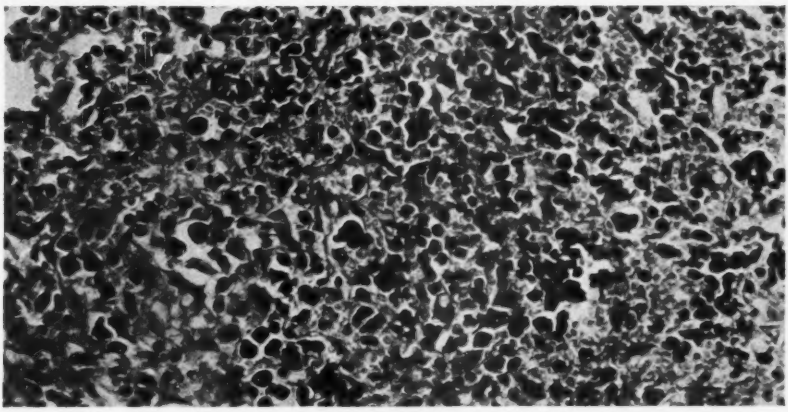

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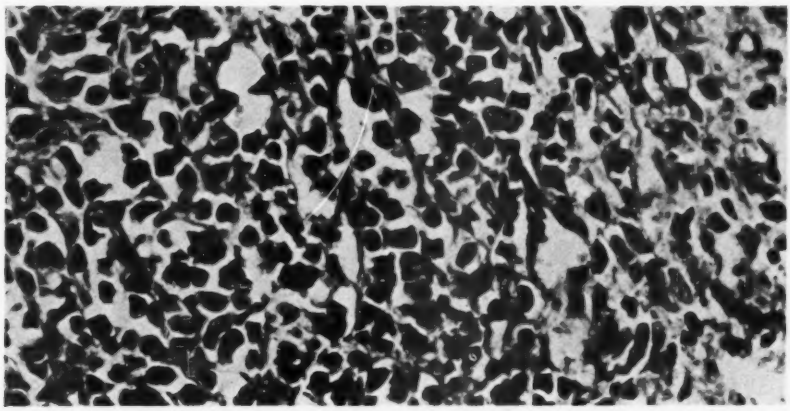

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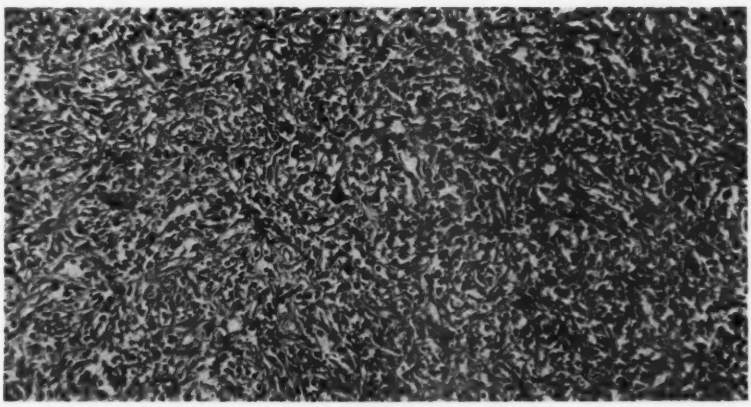
PLATE 12

FIG. 17. Case 2. Photomicrograph showing the reticular scarring of the spleen with residual cellular components of the more diffuse granulomatous reaction typical of advanced lesions (× 117). A comparable view of the spleen of case 1 is shown in Figure 15. (See also Figs. 3 and 4.)

FIGS. 18 and 19. Case 3. Figure 18 shows the margin of a densely scarred nodule in the spleen (× 315). The cells at the center are eosinophilic leukocytes; the spaces in the tissue appeared to have been filled with crystals. Figure 19, a similar nodule at lower magnification (× 147), shows on the left the relatively acellular character of this old and presumably nearly healed granulomatous focus. It is possible to trace a direct relationship between the obvious granulomatous reaction (Figs. 14, 15, and 17), the focal tumor-like reticulo-endothelial proliferation (Fig. 16), and the fibrous healed lesions shown in these two photomicrographs.



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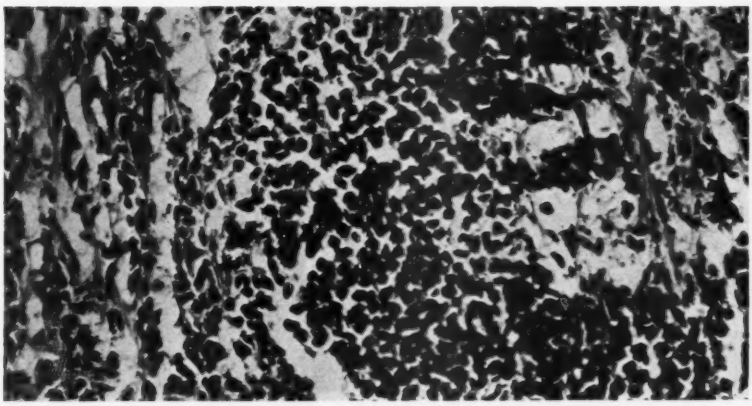

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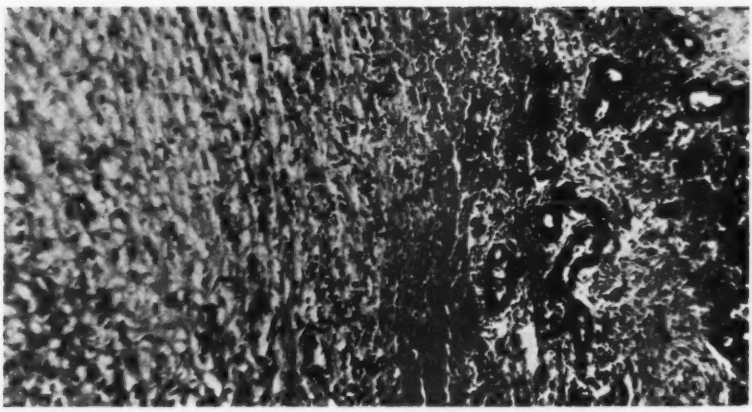

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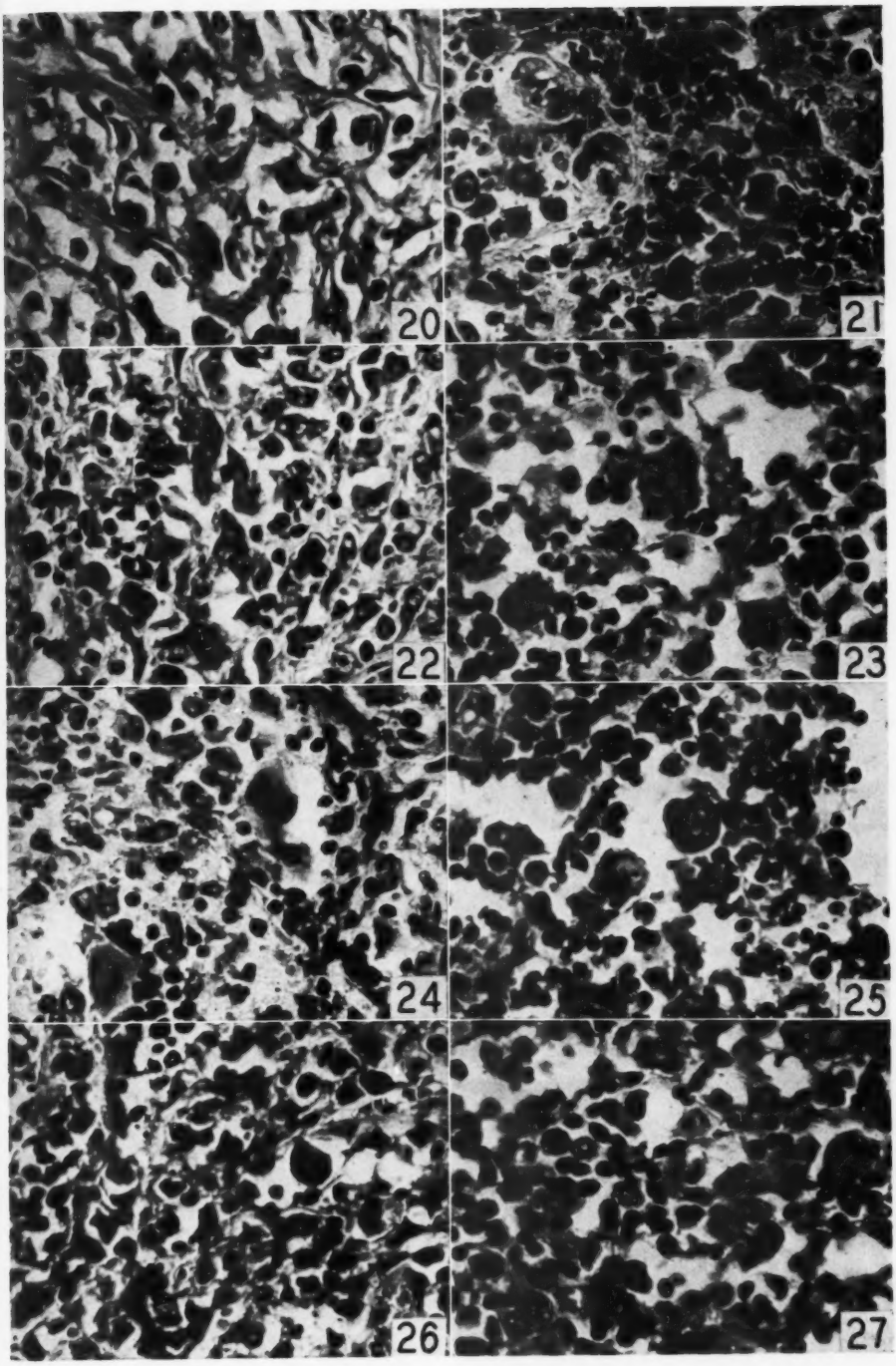
PLATE 13

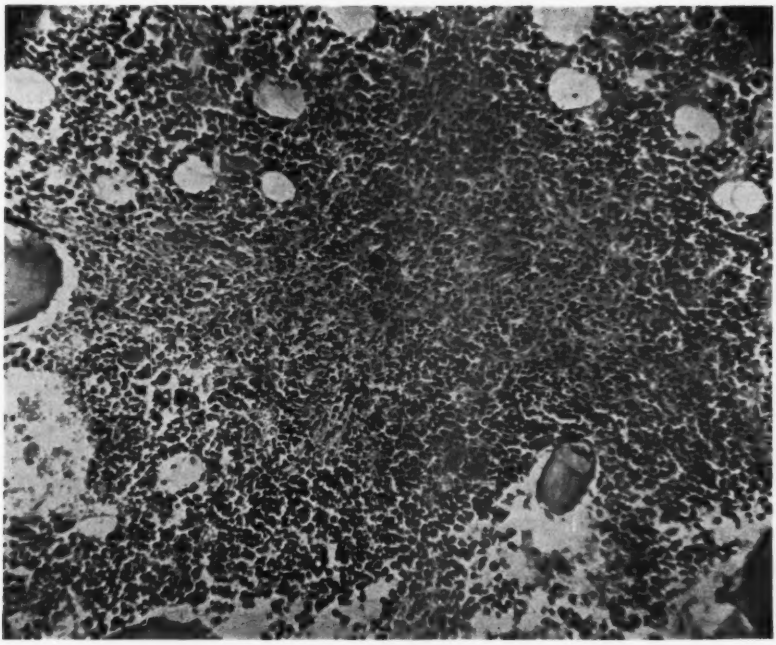
FIGS. 20 to 27. These figures show typical cytologic details of the more active stages of the granulomatous reaction which characterizes the lesion of the spleen in the Hodgkin's-like disease of the hog. Figure 20 is from case 2 (Figs. 4 and 17); Figure 21 is from case 6 (Fig. 5); and Figures 22 to 27 are from case 4 (Fig. 2). All are at the same magnification (× 485) and constitute a typical composite picture of the granulomatous lesion characteristic of the spleen.



Forbus and Davis Disease of Swine Resembling Hodgkin's Disease

PLATE 14

FIGS. 28 and 29. Case 1. The bone marrow lesion is shown in a more active part of the typical granulomatous reaction (Fig. 28, × 137 and Fig. 29, × 345). (See also Fig. 6.)



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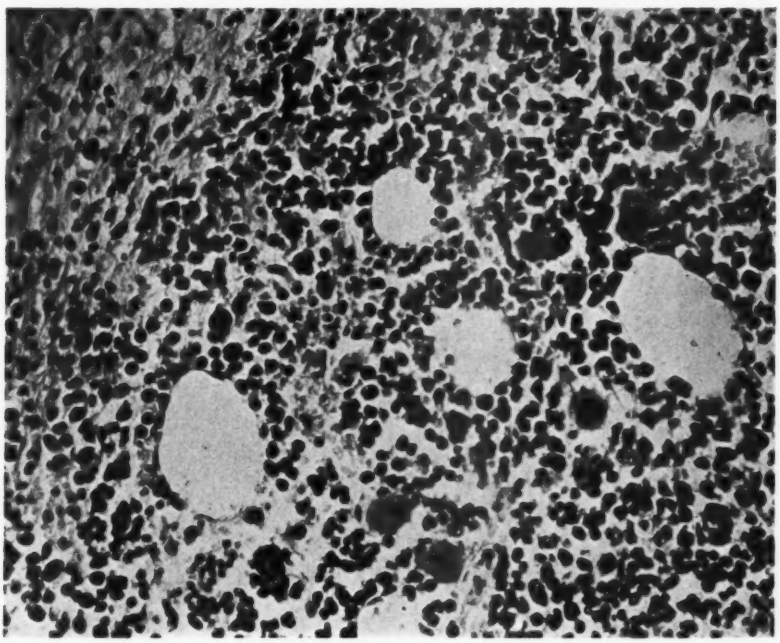

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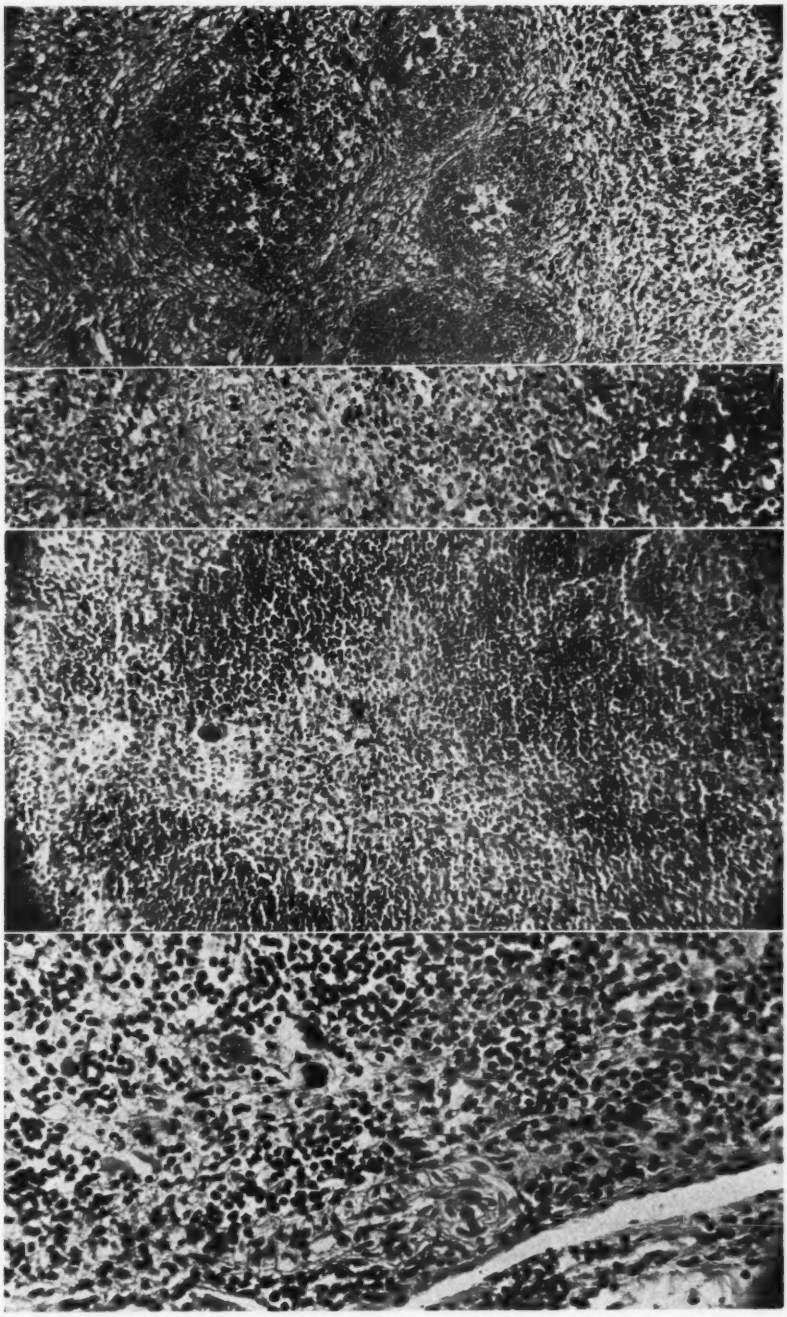
PLATE 15

FIGS. 30 to 33. These figures show a composite picture of the granulomatous lesion of the lymph nodes (capsule at bottom, medulla at top) characteristic of the Hodgkin's-like disease of the hog. Figure 30 (× 122) is from case 1 (Fig. 1); Figures 31 (× 137) and 33 (× 265) are from case 4 (Fig. 2); and Figure 32 (× 112) is from case 5.



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FAT EMBOLISM *

COMDR. SHIELDS WARREN, (MC) USNR

(From the Army Institute of Pathology, Army Medical Museum, Washington 25, D.C.)

Fat embolism, chiefly a pathologic curiosity in time of peace, is a problem of real clinical importance as a result of the inevitable increase in trauma associated with war. Although a considerable amount of animal experimentation has been carried out on the subject, interpretations of results are conflicting. Thus far no effective therapy is available. Consequently a clear understanding of the pathology of the condition, including its pathogenesis, is essential to minimize its occurrence and to determine more accurately its prognosis.

Fat embolism in man is due to fat gaining access to the circulation, either intrinsic fat freed through trauma or extrinsic fat or oil introduced into the organism for therapeutic or other purposes.

In an effort to determine the military importance of fat embolism and the factors favoring the development of the lesion, I have reviewed 100 consecutive cases of this condition in the files of the Army Institute of Pathology. These cases cover a period of approximately 4 years, from the time of large-scale mobilization to the latter part of 1944. The patients were chiefly young, healthy males. Ninety-one cases were due to trauma with fracture of one or more bones; 4 cases were due to blast injury without fracture; 3 to burns; and 1 each to pressure asphyxia without fracture, and to trauma from shell fragments.

The localization of the fractures in these 91 cases was notable. Some cases had fracture of only a single bone and others had multiple fractures, but the tibia, the femur, or both combined were involved in 82 per cent of the cases of fat embolism following fracture. This is perhaps due to the large reservoirs of fat they contain. In reviewing cases of fat embolism reported in the literature, the frequency of fractures of these bones is also apparent.

The means of trauma were varied. Motor vehicles produced injuries in the greatest number of cases (61). Horse, mule, street car, parachute, and glider each provided 1 case.

All but one of these cases occurred in males. The age distribution shown in Table I is about that which would be expected from army personnel, 59 per cent of the cases falling in the age group 18 to 25 inclusive.

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The total amount of body fat has little relation to the occurrence of fat embolism. The state of nutrition or the body weight was specified in 85 cases. Of these, 62 were within normal limits and 23 were overweight. Physical selection would tend to keep the strikingly obese and the emaciated out of the service.

The blood pressure is not a factor. The blood pressure recorded before treatment ranged from none in cases of severe shock to 150/80 mm. Hg. The blood pressure after the onset of symptoms ranged from 0 to 160/90 mm. Hg in all but one case, in which the systolic pressure rose to 192 after the onset of symptoms.

Undoubtedly the state of the circulation is of some importance in influencing the severity of fat embolism, a patient with impaired circulation naturally feeling the effects of a given degree of pulmonary fat embolism more than a patient with good circulation.

TABLE I

Age Distribution of 100 Cases of Fatal Fat Embolism

Years of age	Number
18 — 20	15
21 — 25	44
26 — 30	17
31 — 35	8
36 — 38	6
Unknown	10

The suggestion has been made that status lymphaticus is a predisposing factor in pulmonary embolism. In the course of some 7,000 autopsies, a number of them cases of sudden death, I never have seen an instance of death that could be attributed to status lymphaticus. In light of this and of the work of Farber,[1] Boyd,[2] and others, I doubt that such a diagnosis is ever justified.

There is virtually complete agreement as to the essentials for entry of the embolic fat into the circulation: mobilized fat, veins disrupted and patent, and local pressure.[3,4] Particularly in bone trauma are these factors operative. Fat is set free from the marrow cells. Disruption of veins and venules without opportunity for collapse of their walls is the rule. As hemorrhage, edema, and exudation develop about the fracture, the local pressure is greater than the venous pressure. Consequently it is easy for fat to find its way into the disrupted patent venous channels. Any mobilization of a fracture after local swelling has occurred is fraught with hazard, as a considerable degree of local pressure then exists. Disturbance of the region may dislodge the fibrin occluding disrupted veins, opening them to the free fat present.

In concussions or burns, fat embolism is somewhat more difficult to explain. In marked concussions, as bomb explosion, the extensive disruption of the adipose tissues will very easily force fat into the circulation. In the case of burns, the death of fat cells in the panniculus

may liberate fat and local edema beneath an eschar or a pressure bandage will tend to force the fat into the capillary or venous circulation.

There has been much discussion of the importance of the amount of fat in the blood in relation to embolism. However, the physical state of the fat is probably much more important than is its amount. The normal blood fat varies from 0.6 to 0.7 per cent.[5] The mere presence of even very large amounts of fat in the blood stream will not produce embolism. Thus in diabetic coma the total blood lipid may reach 20 per cent. Although rare instances have been reported of death from fat embolism in diabetic coma, I have not seen such a case among several hundred patients with diabetic coma. The fat is finely divided as chylomicrons and the number of these tends to increase with the total lipid.[6] So long as the fat remains thus divided it is not dangerous. There is no convincing evidence that chylomicrons coalesce to form masses able to produce embolism. Wilson and Salisbury[7] suggested that dark-field study of the blood is worth while to determine the size of the chylomicrons in cases of suspected fat embolism. They stated that globules up to 12 μ in diameter will go through the pulmonary capillaries.

Curiously enough, in fat embolism the level of total blood fat may not be at all high. While blood fat determinations were run in only a few of the cases in the present series, these were not abnormal.

The fat present in embolism is evidently still fluid, as it can be forced into various shapes to conform to the vascular beds and often can penetrate the finest capillaries. If autopsy is done when the body is still warm, the fat is fluid.

The estimates of the minimal lethal amount of fat entering the circulation of man range from 12 gm.[8] to 120 cc.[9] However, Lehman and Moore, from whose work the larger value is quoted, carried out their experiments on the dog, using cottonseed oil. Since there are wide ranges in tolerance to damage of various types between man and animals, it is unsafe to argue on the basis of their data, which are not in keeping with clinical experience. As little as 2 cc. of paraffin oil used as a lubricant in urethral dilatation has been blamed for embolic death, but the evidence here was not decisive.

Harris, Perrett, and MacLachlin[10] found the minimal lethal dose of human fat for rabbits to be 0.9 cc. per kg. when given in one dose. A much larger amount could be administered without producing death if the dose was divided. Hydrolyzed human fat was much more toxic, the minimal dose being 0.07 cc. per kg. They believed that the tissue lipase splits the fat and that the fatty acids produced by hydrolysis irritate the tissues.

In several cases which I studied the reaction to Nile blue sulfate showed the fat present in even markedly edematous lungs to be neutral fat and not hydrolyzed (Fig. 3).

Vance [11] found 4 to 60 cc. of fat at the site of fracture in bones of the lower extremity. Flick and Traum [12] studied the fat content of arterial and venous blood soon after trauma and found significant increases as early as 10 minutes after injury. Scriba [13] found fat present in the urine of 80 per cent of his cases 2 to 6 days after trauma to bone. Occasionally the fat will disappear from the urine transiently, to reappear later.

The cases studied in this series demonstrate that the fracture of a single bone may, under appropriate circumstances, release enough fluid fat into the circulation at a sufficiently rapid rate to produce death.

The highest level of blood lipid that I have seen recorded was in a case of carbon tetrachloride poisoning reported by MacMahon and Weiss.[14] In this case the blood obtained from the pulmonary artery at autopsy showed 64 per cent total lipid by volume and the blood from the upper end of the inferior vena cava showed 25 per cent. Specimens of blood taken from other portions of the body showed no gross fat. Extensive necrosis of the hepatic cells caused by carbon tetrachloride liberated large amounts of fat in the disorganized hepatic tissue and it was thus easy for it to enter the sinusoids and hepatic veins. The sudan stain of sections of the lungs showed many of the smaller branches of the pulmonary artery completely filled with fat, and congestion and focal hemorrhages. Sudan stains of the other organs showed minute droplets of fat in the vessels of the systemic circulation. In addition to visible fat within the glomerular capillaries, fatty casts were present in the collecting tubules. While death was not ascribed by the authors to pulmonary fat embolism, in view of the high degree of cholemia and the extensive necrosis of the liver, one cannot help but wonder if embolism may not have been an important factor.

Figures vary greatly as to the incidence of fat embolism. Darrach [15] reported 2 cases of fat embolism among 12,000 fractures treated at the Presbyterian Hospital in New York. Gröndahl [16] reported 1 case of fatal cerebral fat embolism among 1,026 fractures. Wilson and Salisbury [7] reported 8 cases, 6 of which were fatal, among 1,000 battle casualties of World War II. Robb-Smith [17] studied 789 consecutive (bombing) accidents, of which 125 were fatal. Forty-one of the fatal cases showed gross pulmonary fat emboli and in 29 pulmonary embolism was a major factor in the deaths. In the group of 125 fatal cases, 12 showed no bony injury and 6 of these were due to fat embolism. On the basis of this he suggested that pulmonary embolism

may be an important factor in death from bombings. Wakeley [18] found fat embolism present to varying degrees in 40 per cent of fatally burned cases. In 100 consecutive autopsies, Wright [19] checked on the presence of fat in the pulmonary vessels. In 52 some degree of fat was present on microscopic examination. Globules of fat were present in every capillary in 3 cases, a globule in every low-power field appeared in 1, and the remainder showed only slight amounts of fat.

While attempts have been made to relate specific symptoms to specific localization in the vascular bed, these have not been very satisfactory: first, because with a severe degree of fat embolism more than one viscus is usually involved and, second, because the pulmonary edema and congestion associated with fat embolism lead to a very frequent masking of other symptoms by the obvious respiratory embarrassment and anoxia.

Of special interest is the existence of a period free from symptoms following trauma in many cases. This free period varies and its close is frequently foreshadowed by a feeling of marked apprehension on the part of the patient. This feeling of apprehension has also been reported in cases of pulmonary embolism of thrombotic or other origin.

Fever is of inconstant occurrence and may be related to disturbance of cerebral function, to infection in the wound, or to the development of pneumonia in the edematous lung. The diagnosis is an especially difficult one at which to arrive and frequently autopsy alone will reveal the cause of death. Physical examination or roentgenography is of little or no help. The occurrence of pulmonary edema in a case of recent trauma should lead to the suspicion of fat embolism and at the end of 36 hours examination of the sputum for fat globules may prove positive. Lipuria may be present but is a rare finding. Examination of the blood for fat is probably of little value.

The gross and microscopic evidences present a wide range of variation. In cases of rapid death there may be nothing apparent grossly beyond an excess of oily droplets in the pulmonary circulation. In the acute cases there is usually accompanying pulmonary edema and in the brain there may be petechial hemorrhages, as a rule perivascular. In cases of longer duration there may be consolidation of the lungs in addition to edema and hemorrhage; in the brain there may be small foci of softening, often surrounded by a hemorrhagic zone. In the pulmonary forms, evidences of asphyxia may be present: marked congestion of the chest, neck, and head; petechial hemorrhages, which occur in order of frequency in the pericardium, the conjunctivae, and the pleurae. Petechial hemorrhages may also appear in various tissues as a result of occlusion of small vessels by the droplets.

On microscopic examination, fat droplets are detected in the small vessels and capillaries of most tissues. These predominate in the lungs and in the glomerular tufts of the kidneys. The size of the droplets varies from a few μ in diameter to 1 cm. or more. The shape of the occluding droplets varies from spherical to elongated cylinders, the shape being determined by the three factors of vessel caliber, surface tension, and circulatory pressure. On becoming freed, by section of the tissue in which they lie, they coalesce rather freely, indicating that no definite membrane is formed about the fat but rather that it is merely coarsely and irregularly suspended in the blood. Nothing that I have seen in these sections would indicate that there has been any appreciable breakdown of the fat or that the fat was in any way toxic to either vascular endothelium or formed elements of the blood.

To detect fat embolism microscopically in routine autopsy material it is well in the ordinarily stained paraffin section to examine the glomeruli and the lungs. If there are a considerable number of vacuoles within the capillaries of the tuft or if some of the alveolar capillaries show vacuoles, it is well to do a fat stain on frozen sections of fresh or autopsy material. It is important that the section should be cut rather thicker than is usually the case, say 30 to 50 μ, inasmuch as the droplets of fat are easily dislodged.

Figure 1, a routine paraffin section, is an example of the extreme degree of vacuolization that can occur in a glomerular tuft due to the lodging of fat in it. Figure 2 shows the sudan stain on a formalin-fixed, frozen section. Figure 7 is a routine paraffin section of the lung with readily apparent vacuoles within the pulmonary vessels. Figure 4 is a frozen section counterpart, stained specifically for fat, showing occlusion of capillaries.

Bürger [20] suggested distinguishing three forms of the pulmonary type of fat embolism: (1) the peracute, where death occurs in a few seconds; (2) the acute, in which the symptoms appear immediately after injury and death occurs in hours to days (the type usually confused with pulmonary edema); (3) the subacute, where a free period intervenes between trauma and the onset of symptoms. The cases studied by me can be grouped into these three types (Table II).

The average time from the onset of symptoms to death in this series was 53 hours and the longest duration, 25 days.

In 3 cases of this series, the origin of the fat from bone was obvious since the fat in the pulmonary vessels was accompanied by fragments of bone marrow.

Although Bergmann [21] noted in 1863 that acute pulmonary edema was often associated with fat embolism, inadequate emphasis has been

placed on this point. The present study brings out the importance of pulmonary edema, often combined with congestion or focal hemorrhage, in the clinical course. Occlusion of large or small branches of the pulmonary artery or pulmonary capillaries leads inevitably to impairment of the endothelium and its supporting cells with increased endothelial permeability so that large amounts of fluid may escape into the lungs.

No sooner has an appreciable amount of the pulmonary circulation been impaired than partial local anoxia begins to develop. Infarction or necrosis of pulmonary tissue seldom occurs since even in extensive and prolonged fat embolism there is a sufficient degree of blood flow kept up by the bronchial arteries and uninvolved portions of the pulmonary tree to prevent actual cell death. However, focal or petechial hemorrhages may appear at regions of vascular occlusion. The local anoxia induced soon leads to an increased permeability of the vascular and alveolar walls so that escape of intravascular fluid occurs into the pulmonary tissues and alveoli. This edema may become exceedingly marked (Fig. 7), interfering further with aeration and thus establishing a vicious circle.

TABLE II
Classification of Cases of Fat Embolism

Peracute cases	9
Acute	17
Subacute	58
Undetermined	16

The average weight of the lungs in these cases, when specified, was 1600 gm. As a general rule, the few cases in which the lungs were relatively free from fluid were peracute or acute cases of short duration in which sufficient time for the development of edema had not been present.

There has been some speculation as to the induction of arterial spasm by the presence of the fat and further production of anoxia by this means. There is no satisfactory experimental evidence for this and the general experience with anoxia in shock and other conditions is that there is a tendency to vasodilatation in the viscera rather than vasoconstriction. The present data do not support the view that such arterial spasm occurs.

Within a few hours after lodging, the fat in the pulmonary circulation begins to gain access to the alveoli and is visible within them, usually as small droplets ranging up to 15 to 20 μ in diameter. These at first are merely dispersed in the edema fluid which accumulates within the alveoli (Fig. 8), but later they are to some degree phagocytized by mononuclear cells, in some instances they become incorporated as vacuoles within the substance of foreign body giant cells. In

view of this phagocytosis, it is not surprising that in those patients living several days after the onset of symptoms of embolism, one will encounter some phagocytized fat in the pulmonary parenchyma. In several of the cases in which the patients had lived for some days, I found fat within phagocytes in mediastinal lymph nodes.

After some 36 hours fat may be observed in the sputum.[22] Staining the sputum of a case of supposed pulmonary edema for fat may disclose fat droplets and thus establish the diagnosis.

Undoubtedly some of the fat may escape from the vessels by actual disruption of their walls. Much of it, however, is present in alveoli in which the capillaries are intact. The cement substance of endothelium and of many other tissues has a lipoidal content which may permit fatty substances, under suitable circumstances, to pass through an intact vessel wall.

TABLE III
Severity of Pulmonary Fat Embolism

Fat			Marrow
+	++	+++	
4	34	62	3

The severity of pulmonary fat embolism in the present series of cases is given in Table III, which shows that in nearly two-thirds of the cases, lodging of fat to a severe degree occurred. Thirty-four patients presented respiratory symptoms as the most striking clinical feature.

While it has been suggested [23] that there is marked renal damage with fat embolism, there is no evidence to support this in my material. In spite of the almost constant involvement of the renal glomerular capillaries (Table IV), the clinical significance is minimal. There is little need to alter the statement made by Warthin [3] in his classic monograph that "Nephritis has never been mentioned as a complication." One can but wonder why the renal glomeruli do not show evidence of damage even when there is virtually complete occlusion by fat of the afferent arterioles as well as all of the capillaries in the glomerular tufts (Figs. 1 and 2). Although involvement of the glomerulus is a frequent feature of fat embolism, I did not encounter in this series a single death due to impairment of renal function. Evidently, either death comes before noticeable renal change can occur, or the damage, if any, is soon repaired. Perhaps the intermittent character of the renal glomerular circulation may have prepared the cells of the tuft to withstand periods of anoxia, and perhaps some tufts, nonfunctioning at the time fat droplets were plentiful in the blood stream, escape embolism.

Lipuria as a means of demonstrating fat embolism is not reliable. It rarely develops before the fourth day. In the majority of the cases in the present series, the urine was not examined for fat. Many pa-

tients died prior to the time when lipuria might have been expected. In some cases it was possible after death to demonstrate fat droplets within the lumen of the tubules (Fig. 5). Rarely, numerous fat droplets were present in the tubular epithelium.

Twenty-four patients died with symptoms predominantly cerebral. The gross and microscopic lesions of the brain have been well studied in the past.[4] The reported findings are confirmed by the present observations. The classical picture is the appearance of small petechial hemorrhages in the leptomeninges as well as throughout the brain, and minute foci of softening scattered indiscriminately in the cortex and

TABLE IV
Renal Abnormalities with Fat Embolism

	Fat in glomeruli				Fat in tubules	Tubular degeneration
	None	+	++	+++		
Number of cases	17	11	15	20	4	0

medulla. There are varying degrees of minute focal hemorrhage. Schmidt [24] used the rather good descriptive term of "purpura cerebri" in this condition. The transition from the gray matter to the white may be indistinct. At times the emboli excite no reaction (Fig. 6).

Microscopically, early vascular occlusion may occur without reaction, or there may be perivascular hemorrhage (Fig. 9), or chiefly perivascular edema (Fig. 10). Lesions of longer duration may present small foci of degeneration (Fig. 11) adjacent to a vessel occluded by fat, and sometimes there are small anemic infarcts with a hemorrhagic periphery similar to that seen in anemic infarcts otherwise produced. A fair number of polymorphonuclear leukocytes are present in the earlier stages of the lesion. These gradually degenerate and mononuclear phagocytes take their place. The infarcts may be anemic in the gray matter, hemorrhagic in the white.[25] Changes have been described in the neurons themselves but it is very difficult to differentiate between the effect of the general anoxia on them and the effect of local embolic occlusion. Advanced lesions are rarely seen since death, if it occurs, tends to appear fairly soon after cerebral involvement.

Trauma to the head had occurred in 33 cases. There was no correlation between this and fat embolism of the brain.

While fat embolism of the central nervous system occurs frequently (31 of 100 cases in the present series), there is no correlation between it and pulmonary embolism other than the fact that cerebral embolism, of course, cannot occur without some degree of pulmonary involve-

ment. Cerebral fat embolism may be found at autopsy in a slight or moderate degree without there having been presented any evidence of its occurrence during the life of the patient. In this present series, a slight amount of fat was present in 7 cases, a moderate amount in 13, and severe involvement in 11.

A rapid rise in temperature without evidence of pneumonia suggests that the brain has been involved by the embolic process. Among the few patients with recognized fat embolism who have recovered, residual mental or neurologic changes have not been noticed. The character of the lesions as seen in some fatal cases, however, would suggest that such changes will eventually be found.

Since fat which passes through the pulmonary circulation or through a patent foramen ovale may lodge anywhere in the systemic circulation, one finds at times very widespread emboli. It is questionable whether embolic involvement of coronary vessels may be a significant factor in producing death. In cases where the coronary arteries are heavily involved there is much embolism elsewhere. In the present series I encountered 8 cases in which an apparently significant degree of coronary involvement was present. One case of the series showed a patent foramen ovale, but the clinical picture and the distribution of fat did not differ from those of the others.

Owing to the lodging of small emboli in the vessels of the skin or mucous membranes, petechiae may develop [26] (Fig. 12). At autopsy, of course, pericardial and pleural petechiae associated with the anoxia produced by the pulmonary embolism are of frequent occurrence.

Rarely, changes may be noted in the eyegrounds, but these are usually degenerative in type [27] rather than hemorrhagic, and probably result from embolic occlusion of the nutrient vessels.

Unfortunately, the nature of the condition is such that no specific therapy for fat embolism exists, and such measures as are applied are usually futile. In general, supportive treatment is about all that can be attempted.[28] Once the pulmonary form has been recognized, the use of oxygen may be advantageous to prevent further development of anoxia and pulmonary edema.

In the way of prophylaxis one can do little but reiterate the suggestions already made many times that fractures be immobilized to the greatest possible degree and that any manipulation of them be carried out either before appreciable local swelling has occurred or after it has subsided.

Summary and Conclusions

1. The pathologic findings in 100 fatal cases of fat embolism have been summarized. Ninety-one cases followed fracture of one or more bones.

2. Fractures of femur, tibia, or both, either alone or combined with fractures of other bones, were antecedent to fat embolism in 82 per cent.

3. Fat embolism may also follow extensive trauma to soft parts; for example, through bombing, burning, or crushing.

4. Fat embolism is not due to aggregation of circulating fat, but to quantities of fat set free from the bodily deposits, or rarely, to fats introduced from without.

5. Fat embolism in the present series of cases produced instant death (peracute form), in 9, death following symptoms of immediate origin (acute form) in 17, or death following symptoms developing after a free period (subacute form) in 58 cases.

6. The occurrence of fat embolism is apparently independent of the nutritional state.

7. A free period in which the patient may be apprehensive may precede the development of symptoms.

8. Fat embolism may be recognized clinically by the development of pulmonary or cerebral symptoms following (a) a fracture or extensive injury of soft parts; (b) manipulation of a fracture. After some time, lipuria or fat droplets in the sputum may aid in establishing the diagnosis.

9. Although renal fat embolism may be marked, renal failure is not a cause of death.

10. In many cases fat embolism will be missed as a cause of death unless it is searched for consistently.

11. In autopsy material the presence of fat may be easily checked by watching for vacuoles in the glomerular tufts or in the lung parenchyma in routine sections.

12. Therapy is usually unavailing and is best directed toward the prevention of anoxia.

I am indebted to the staff of the Army Medical Museum for help, without which the publication of this study would have been impossible. In particular, I wish to thank the Photographic Department which prepared all of the illustrations.

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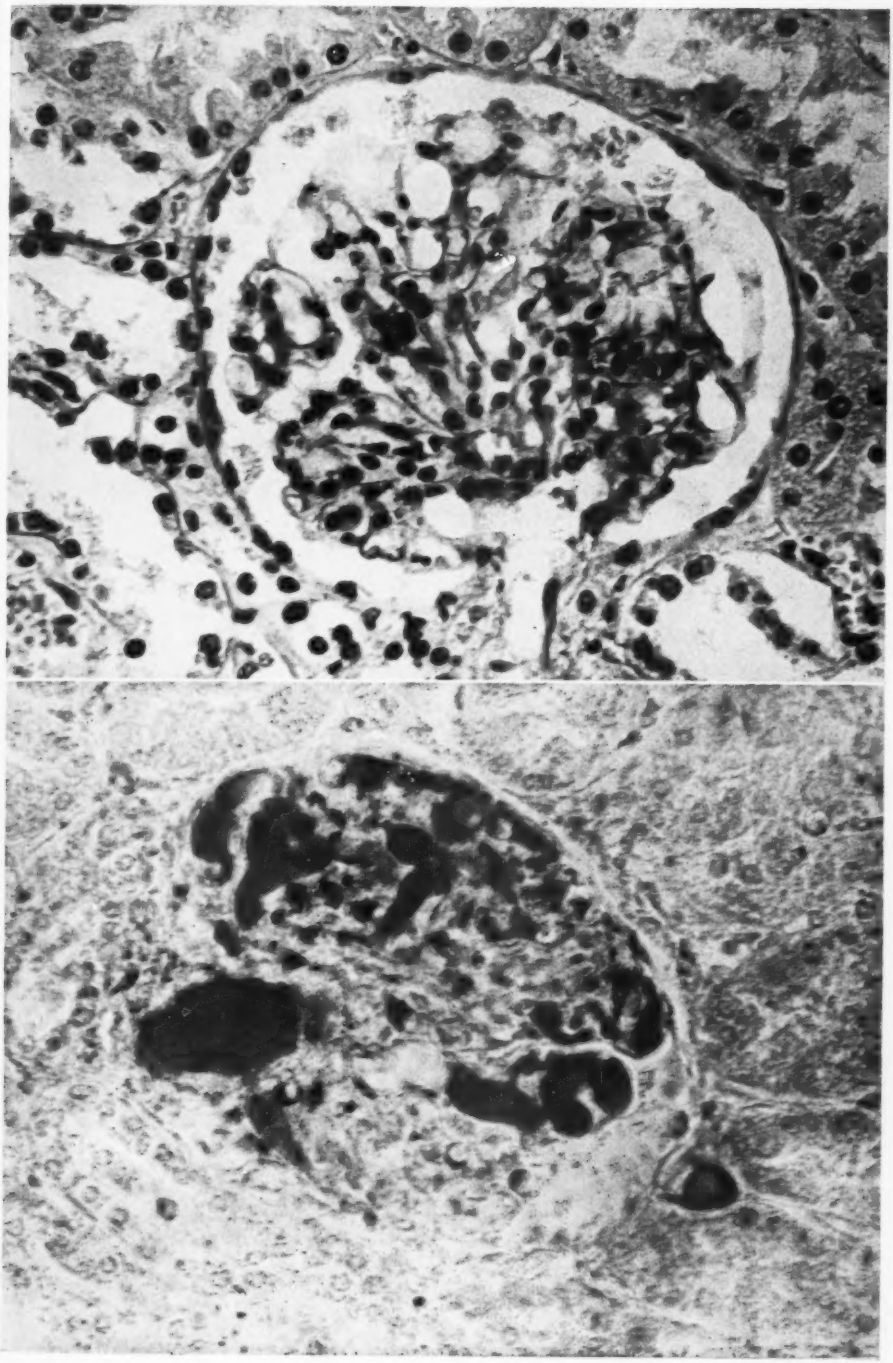
DESCRIPTION OF PLATES

PLATE 16

FIG. 1. Paraffin section of glomerular tuft showing vacuolization of capillaries and arteriole due to embolic fat. Case 118020. Hematoxylin and eosin stain. × 500.

FIG. 2. Frozen section of glomerular tuft showing vacuolization of capillaries and arteriole due to embolic fat. Case 117306. Sudan IV stain. × 500.



Warren Fat Embolism

PLATE 17

FIG. 3. Pulmonary fat embolism showing neutral fat in blood vessels and in edematous alveoli. Case 118020. Nile blue sulfate stain. × 500.

FIG. 4. Fat occluding alveolar capillaries. Case 114289. Sudan IV stain. × 160.

FIG. 5. Fat present within the lumina of renal tubules. There is no evidence of injury of the tubular epithelium. Case 117306. Sudan IV stain. × 500.

FIG. 6. Embolic occlusion by fat of capillaries in the white matter of the cerebrum. No reaction is present. Case 117306. Sudan IV stain. × 230.

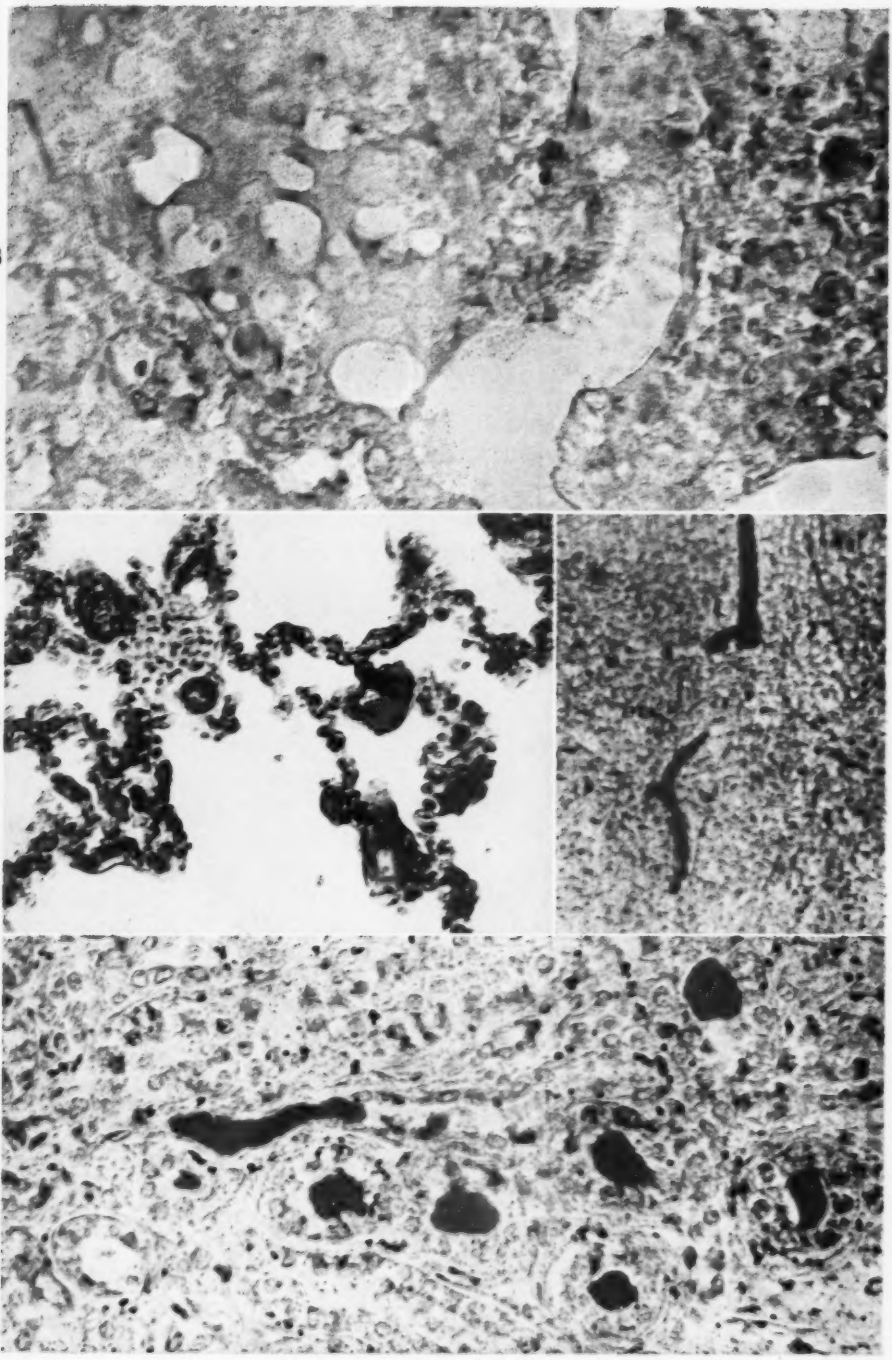

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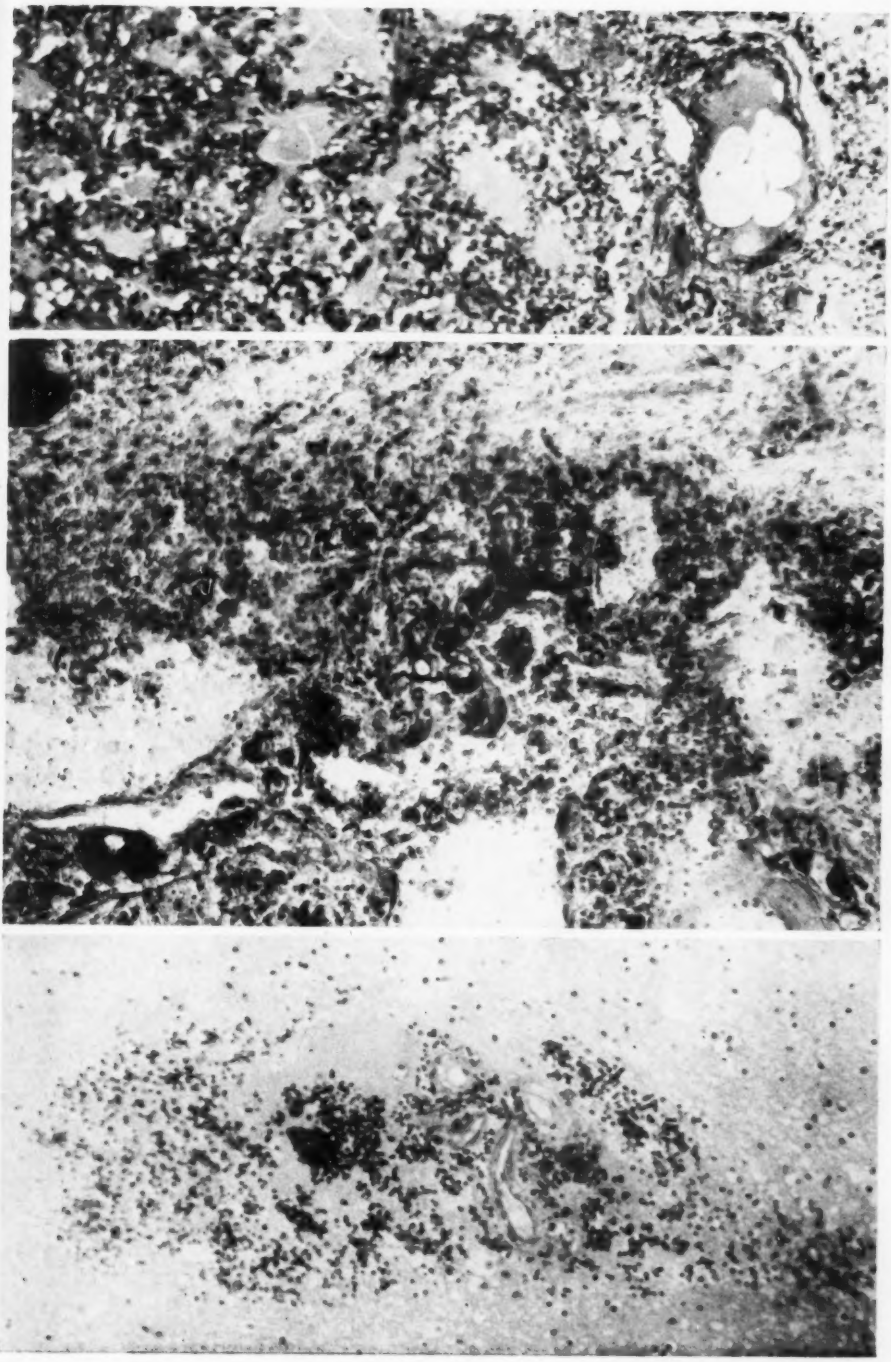
Plate 18

Fig. 7. Fat droplets within pulmonary arteriole. showing alveolar edema and small fat droplets. Case 118020. Hematoxylin and eosin stain. × 145.

Fig. 8. Fat droplets in pulmonary vessels and in edema fluid within alveoli. Case 118020. Sudan IV stain. × 145.

Fig. 9. Perivascular hemorrhage about arterioles occluded by fat. Case 99570. Hematoxylin and eosin stain. × 145.



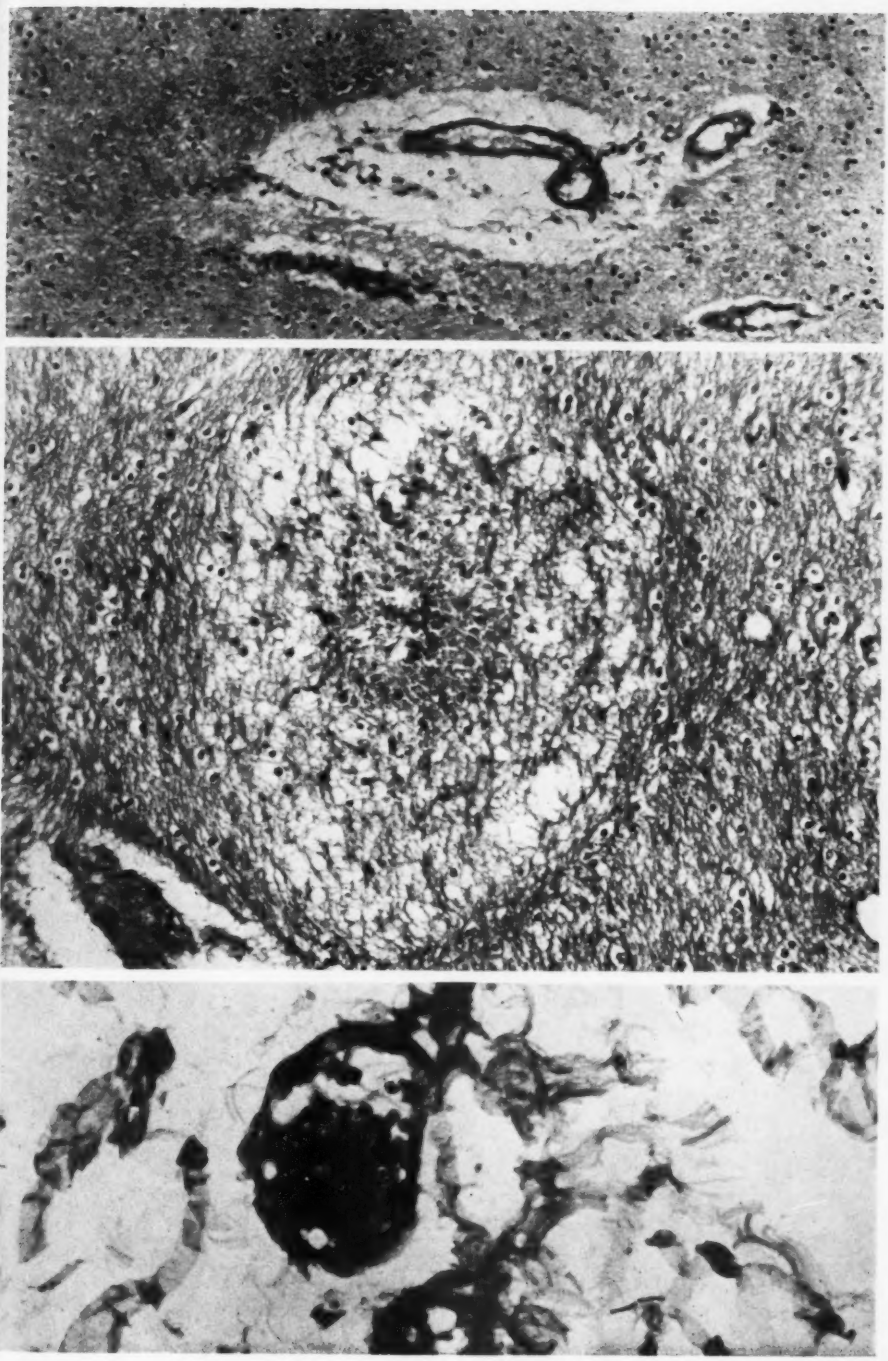
Warren Fat Embolism

Plate 19

Fig. 10. Perivascular edema in the white matter of the cerebrum secondary to fat emboli. Case 118020. Hematoxylin and eosin stain. × 145.

Fig. 11. Focal demyelinization in the white matter of the cerebrum, resulting from embolic occlusion of small arterioles by fat. Case 100989. Hematoxylin and eosin stain. × 230.

Fig. 12. Capillaries in panniculus adiposus, partly occluded by fat droplets. This case showed petechial hemorrhages of the skin. Case 117306. Hematoxylin and eosin stain. × 500.



Warren Fat Embolism

GROWTH OF THE RICKETTSIAE OF TSUTSUGAMUSHI FEVER ON THE CHORIOALLANTOIC MEMBRANE OF THE DEVELOPING CHICK EMBRYO*

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The rickettsiae of tsutsugamushi fever (*Rickettsia orientalis*) can be cultivated in the yolk sac of the developing chick embryo in the same manner as the other rickettsiae (Lewthwaite and O'Connor, 1943). This has been confirmed by others as well as by me. It was of interest to test the growth potentialities of tsutsugamushi rickettsiae on other media, including cultivation on the chorioallantoic membrane of the developing chick embryo, especially since it had been shown that the rickettsiae of epidemic typhus could be grown on this membrane (Zia, 1934). In contrast with the sparse growth of epidemic rickettsiae, when the organisms of tsutsugamushi fever were inoculated onto the chorioallantoic membrane a large ulcerated and nodular lesion was produced which thus far has developed in essentially its original form throughout 26 successive transfers.† The magnitude of the response elicited in the host tissue and the presence of numerous rickettsiae in smears from the lesion prompted me to make a more detailed study of the development and histologic character of the nodules. The results, which are presented here, are of interest because of the remarkable proliferative response induced in the host tissue by the presence of tsutsugamushi rickettsiae.‡

METHODS

The initial lesions were produced by inoculating peritoneal exudate from infected mice, obtained when the animals were moribund, onto the chorioallantoic membrane of 10-day chick embryos. The peritoneal exudates were obtained from mice that had been infected with two strains of tsutsugamushi fever which had been isolated recently in New Guinea.§ These are known as the Kostival and Shope strains. Each

* Received for publication, June 2, 1945.

† The use of the chorioallantoic membrane as a site for growing tsutsugamushi rickettsiae was mentioned by Lewthwaite and O'Connor (1943). In this report, they stated that it "had given poor results." Recently, I had the pleasure of seeing Dr. Lewthwaite, and he showed me photographs of chorioallantoic membranes which had been infected with scrub typhus. At that time he had made no histological study of the lesions and had not studied their specificity. However, the gross appearance of the lesions was essentially identical with what I had obtained.

‡ This study was initiated at the suggestion of Colonel Harry Plotz, M.C., Chief of the Division of Virus and Rickettsial Diseases, Army Medical School, and member of the U.S.A. Typhus Commission.

§ Strains were isolated by Captain E J. Bell, Sn.C., of the U.S.A. Typhus Commission.

sample of peritoneal exudate was diluted 1:5 with physiological salt solution, and 0.2 cc. amounts were placed on each chorioallantoic membrane. The eggs were then incubated at 37.5° C. for 6 to 7 days. The initial lesions which resulted were removed with sterile technic. Smears from them were stained by Giemsa's method, differentiated in acetone, and examined for rickettsiae. Subsequent egg passages were made by grinding a single lesion in 2 cc. of salt solution and inoculating 0.2 cc. of the suspension on each chorioallantoic membrane. Both strains of tsutsugamushi gave similar lesions.

For histological study, the lesion was clipped from the egg along with a wide area of chorioallantoic membrane and pinned out with glass needles on a layer of wax in a Petri dish. The preparation was then covered with Zenker's solution and fixed for 12 hours. After washing in water, the lesion was trimmed, dehydrated in alcohol, embedded in paraffin, sectioned at 4 μ, and stained by Wolbach's modification of the Giemsa method.

Gross Structure of the Lesion

The chorioallantoic lesions have varied in size from a pin-point thickening to a convoluted tumor-like mass which measured 18 by 11 by 5 mm. The average-sized lesion was 10 by 6 by 3 mm., as shown in Figures 1 and 2. The size of the lesion was influenced by the type and concentration of the inoculum.

At the height of its development (5 to 7 days), the lesion appeared as a large, white, nodular mass. On its external surface (where the inoculum was originally placed) there was typically a yellowish gray ulcer forming a sort of shallow concavity in the flat surface of the membrane (Fig. 1). In some cases this concavity was filled with a droplet of clear, viscid fluid.

The main body of the lesion was best seen by removing the chorioallantoic membrane and turning it over so that the lesion could be seen from the internal surface (Fig. 2). Typically, it consisted of a large, white, thickened nodule or cluster of nodules. However, in rare cases there might be "daughter colonies" in addition to the main nodular mass. Beneath the main body of the lesion was a stringy network of grayish precipitate which was enmeshed in the nodular mass but easily separable from it and which floated partially free in the allantoic fluid.

The nodular mass was well vascularized (Figs. 1 and 2). Whether this occurred through an original localization of rickettsial growth at the juncture of two blood vessels was not known, but it appeared that there must have been a certain amount of subsequent enlarging or diverting of blood vessels to supply the lesion, for many more large

vessels were found there than would be found in a similar area of normal membrane. In some cases, the nodular mass was so well vascularized that it was surrounded by large vessels, radially arranged like the spokes in a wheel.

Smears were made from the exudate above the ulcer, the nodular mass, the gray precipitate beneath the lesion, the allantoic fluid, the embryo, and the yolk sac. Rickettsiae were very numerous in the nodular mass, and sparse in the exudate above the ulcer, but were not found in any of the other places which were examined.

In order to determine the order of infectivity of the chorioallantoic lesion, serial dilutions of a suspension prepared from one lesion were made in the concentrations 1:5, 1:50, and 1:500, and inoculated in 0.2 cc. amounts onto the chorioallantoic membranes of three series of eggs. Typical large, thickened lesions were found in the lowest dilution used, like those obtained in routine passages. With a dilution of 1:50, the lesion was somewhat smaller (diameter of approximately 2 mm.), there was less thickening, and in place of the ulcerated surface there was a central hemorrhagic area. At the highest dilution, 1:500, there was a very small but definite lesion consisting of a whitish, opaque spot (0.5 to 1.0 mm. in diameter) surrounded by a transparent area. Grossly, there did not appear to be any appreciable thickening of the membrane. The lesion resembled the occasional "daughter colonies" which occurred in conjunction with a main nodular mass on membranes that were heavily infected.

When nodules were ground up and titrated in mice by intraperitoneal inoculation, infectivity was shown to be as high as 10^{-7}. The disease induced in the mouse was identical to that produced by passage of other infectious materials.

Histologic Structure of the Lesion

Embryologically, the chorioallantoic membrane is derived from a fusion of the chorion with the allantoic sac, each of which consists of an epithelial layer of cells and a layer of mesoderm. The two membranes are fused so that the mesodermal layers are in apposition. Thus, in section, the chorioallantoic membrane consists of an outer layer of ectoderm (from the chorion), a middle layer of mesenchyme (from both chorion and allantois), and an inner layer of endoderm lining the allantoic sac. Normally, the thickness of the chorioallantoic membrane does not exceed 0.25 mm.

By far the most striking feature of the lesion produced by *R. orientalis* was the tremendous proliferative response evoked in the host tissue. The nodular mass might be 5 mm. thick, or as much as twenty

times the thickness of the normal membrane. Histologically, it was seen that the thickening was due primarily to a proliferation of the mesenchyme (Figs. 3, 4, and 5) and infiltration by ameboid "round cells." The prominent nucleoli and chromatin pattern in these "round cells" indicated that they were probably stem cells (*i.e.*, embryonic leukocytes) (Fig. 8). The outer, ulcerated surface of the lesion was bordered by rounded, partially necrotic cells instead of the normal epithelial layer. Immediately underlying this ulcerated edge was a layer of denser, more deeply staining mesenchymal cells resembling fibroblasts. Within the remainder of the nodular mass, which consisted of an enormously thickened layer of mesenchyme containing many blood vessels, there were centers of necrosis surrounded by a layer of mesenchymal cells of the fibroblastic type (just as underlay the ulcerated surface of the lesion) (Figs. 3, 4, 5, 8, and 9). Large numbers of ameboid stem cells infiltrated the mesenchyme and were aggregated around the necrotic areas (Fig. 8). Eosinophilic leukocytes were also very numerous in the nodular mass, particularly in the lumina of blood vessels. Although typical thrombi were not noted, the aggregation of stem cells and eosinophilic leukocytes often formed a tight mass which completely filled the lumen of the vessel. There was some inflammation of the blood vessel walls, especially within the areas of proliferation and necrosis. The endothelial cells were swollen and increased in number, and the wall of the vessel was often necrotic. The human lesion rarely contains eosinophilic leukocytes, and while phlebitis also occurs, it is not associated with necrosis.* Because of these differences, it appears inadvisable to make a comparison between the chorioallantoic nodular mass and the human eschar.

The development of individual nodules within the chorioallantoic lesion appeared to depend on a local proliferation of the mesenchyme. Each center of proliferation, with its fibroblastic type of mesenchyme, enlarged and spread, and might merge with other centers of proliferation (Figs. 3 and 4). During this stage the nodular mass was formed and became increasingly vascularized. Eventually the cells in the center of each proliferative mass became necrotic (Fig. 5). In cases where the center of proliferation lay near the surface of the nodule, the necrotic mass might break through the epithelium and form a secondary ulcer (Figs. 4 and 5).

In favorable preparations, rickettsiae appeared to lie free in the interstices of the mesenchyme of the entire lesion (Figs. 11, 12, and 13). They were scarce in the centers of the necrotic areas, probably because

* Personal communication from Captain A. C. Allen, M.C., of the Army Institute of Pathology.

of poor conditions for growth, but were particularly abundant around their edges. The stem cells which collected around the centers of infection seemed to be actively engaged in ingesting rickettsiae, for they had short, broad pseudopods, and many were filled with rickettsiae. The rickettsiae themselves, which lay free in the tissue, had the typical diplobacillary form with bipolar granules (Fig. 11). However, after they had been ingested by stem cells they appeared shorter and more densely stained. Both the outer and inner epithelial layers of the chorioallantoic membrane were infected with rickettsiae in the region of the nodular mass (Fig. 10). The organisms were more intensely stained when inside epithelial cells, and were more numerous in the outer layer (on the surface of which the original inoculum was placed) than in the inner (endodermal) epithelium.

In spite of the large numbers of rickettsiae which they contained, the epithelial layers were ordinarily only slightly thickened (Figs. 3, 4, and 5). In some cases, however, the epithelium had been so stimulated to proliferate that large convoluted or papillomatous masses were formed (Figs. 6 and 7).

When infected yolk sac was used as a source of inoculum on the chorioallantoic membrane, the size of the lesion and yield of rickettsiae were greatly increased (a 1:20 suspension by weight was made by grinding the sac and diluting with physiological salt solution; 0.2 cc. of this inoculum was placed on each chorioallantoic membrane). The nodular mass, though larger, did not differ structurally from the smaller type produced when infectious material was obtained from mouse peritoneal exudate.

Specificity of the Lesion

The character and magnitude of the lesion and presence of rickettsiae in it would indicate that it was directly caused by these organisms. However, the chorioallantoic membrane is a particularly good site for growth not only for bacteria but also for fragments of tissue, so that it appeared desirable to test whether the irritation and subsequent nodular mass were produced specifically by the rickettsiae. The possibility that extraneous organisms might be involved was eliminated by making blood agar cultures of the suspensions used for each chorioallantoic passage, and by testing the nodules for infectivity in mice. Lesions were taken from the 5th and 21st passages, ground in salt solution, and injected intraperitoneally into four mice each (1:5 dilution). Material from the 5th passage caused death of all mice in 6 to 9 days; those which received inoculum from the 21st passage died in 5 days. The animals showed typical symptoms of tsutsugamushi disease with many

intracellular and extracellular rickettsiae in the peritoneal exudate. No organisms, other than the rickettsiae, were demonstrable either in the blood agar cultures or in the peritoneal exudates from mice which were infected with nodular material.

The chance that fragments of tissue in the inocula might grow and form chorioallantoic grafts did not appear probable when the source of infectious material was peritoneal exudate from adult mice. When the inocula were obtained from embryonic tissues, however, such as infected yolk sac or chorioallantoic nodules, it seemed very probable that fragments of embryonic tissue might become established as grafts and form proliferative nodules. This possibility was tested by preparing suspensions from normal yolk sac and chorioallantoic membrane in the same way as was done with infected tissues, and inoculating them onto chorioallantoic membrane for the same (7 day) growth period. Sporadic, pin-point colonies of tissue were found on some membranes, but these were infrequent, insignificantly small, and entirely different in appearance from the nodules produced by tsutsugamushi rickettsiae. Furthermore, when tissues infected with tsutsugamushi rickettsiae were repeatedly frozen and thawed so as to destroy all living cells, the inoculum prepared from such frozen tissues produced typical, large, ulcerous nodules on the chorioallantoic membrane. It seemed clear, therefore, that the lesion was not due to the presence of living tissue cells in the inoculum.

A further possibility is that the proliferative nodule may be caused only secondarily by the rickettsiae. The organisms might multiply in the chorioallantoic tissues, causing destruction of cells, and the necrosis *per se* might be sufficient stimulus to form a nodule. Or the rickettsiae might be only one of a number of stimuli which could cause irritation and sufficient tissue reaction to form a nodule. To test whether necrotic tissue was involved, fragments of embryonic tissues (yolk sac and chorioallantoic membrane) which had been killed by freezing, were placed on the chorioallantoic membrane. After 7 days of incubation, the membranes were examined for lesions. In most cases (10 of 11) there was a yellowish white, slightly thickened area where the dead tissue was in contact with the chorioallantoic membrane, but in no case was there a formation of nodules or extensive vascularization such as was obtained when rickettsiae were present. These experiments suggest, therefore, that dead tissue is in itself not a sufficient stimulus for the formation of nodules.

To test the possibility that tsutsugamushi rickettsiae might be only one of a number of stimuli which might lead to the same result, *viz.*, nodule formation, I inoculated different kinds of rickettsiae on the

chorioallantoic membrane. A grayish thickened area was produced on the membrane by fièvre boutonneuse rickettsiae. Large numbers of organisms were seen in smears from this area, and the embryos died of the infection in 3 to 4 days. The rickettsiae of Rocky Mountain spotted fever did not kill the embryos until 4 to 6 days after inoculation. Progressively larger thickenings and nodules were formed, depending on the length of the growth period, until by the sixth day the nodular mass was comparable in size to that produced by tsutsugamushi rickettsiae. However, an ulcerated outer surface was obtained only with *R. orientalis*. Zia (1934) has demonstrated that the rickettsiae of epidemic and Mexican typhus can produce occasional small nodules, although the usual reaction was a simple thickening of the chorioallantoic membrane. His brief description of the nodules suggests that they are similar in structure, although much less extensive, than those I have obtained by using rickettsiae of tsutsugamushi and Rocky Mountain spotted fever. It appears, therefore, that nodule formation, *per se*, is not a specific phenomenon, but may be produced by several kinds of rickettsiae.

Other evidence is available which indicates that the chorioallantoic membrane may respond to many diverse stimuli by producing nodules. Danchakoff (1916) found that when adult chicken spleen (and some other tissues) was grafted on the chorioallantoic membrane, the hematopoietic organs of the host embryo became enlarged. The most apparent enlargement was in the host spleen, and was caused primarily by an intense proliferation of both the mesenchyme and the young stem cells. Centers of necrosis appeared in the spleen as a result of this excessive accumulation of tissue with an inadequate capillary supply. Similar results were obtained by Willier (1924) when small pieces of adult chicken thyroid gland, liver, or thymus were grafted on the chorioallantoic membrane. Large nodular grafts were produced which contained necrotic areas. Histologically, it was found that the grafts were formed in large part by proliferation of the mesenchyme and a tremendous infiltration of leukocytes. In each case, the spleen of the host embryo was correspondingly enlarged and showed a number of necrotic areas in section (Willier, 1924).

I have examined the spleens of infected embryos which had large chorioallantoic nodules produced by the growth of tsutsugamushi rickettsiae, and have found that they, too, are several times normal size. There was greater enlargement of the host spleen when the chorioallantoic nodules were larger. Smears from the host spleen contained large numbers of stem cells and many rickettsiae. Danchakoff (1916) postulated that certain kinds of grafted tissue liberate sub-

stances which stimulate the hematopoietic organs and induce intense proliferation of their cells. Reasoning by analogy, it appears probable that rickettsiae liberate toxins which incite a similar activity of the hematopoietic system, with a mobilization of stem cells at the centers of infection. The resultant chorioallantoic nodule (and enlarged spleen) thus would represent a secondary and nonspecific effect of the growth of rickettsiae.

Summary

1. A large nodular mass of tissue with an ulcerous outer surface is formed by the growth of *Rickettsia orientalis* on the chorioallantoic membrane of the chick embryo. The nodule, which is proliferative in nature and highly vascularized, usually becomes 8 to 10 mm. in diameter and 4 to 6 mm. thick after 7 days of growth.

2. The nodular mass is formed by extensive proliferation of the mesenchyme around centers of rickettsial growth. As each locus of rickettsial growth increases in size, the centrally located cells become necrotic. Embryonic leukocytes—"stem cells"—migrate into the nodule and ingest rickettsiae from the fringe of growth surrounding the necrotic areas.

3. Rickettsiae are found scattered throughout the mesenchyme of the nodular mass, but are particularly abundant around the necrotic areas. They also occur in large numbers in the epithelial layers.

4. A feature of the lesion is the remarkable response of the host tissue to the growth of rickettsiae. There is not only a tremendous proliferation of mesenchyme and the formation of layers of primitive, fibroblast-like cells around loci of rickettsial growth, but in some cases the epithelium also is stimulated to form multi-branched and thickened, papillomatous growths. The formation of nodules on the chorioallantoic membrane is not considered specific for tsutsugamushi rickettsiae. Grossly similar nodules are produced by the growth of rickettsiae of Rocky Mountain spotted fever on this membrane. The size of such nodules is apparently directly correlated with the length of the growth period.

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[*Illustrations follow*]

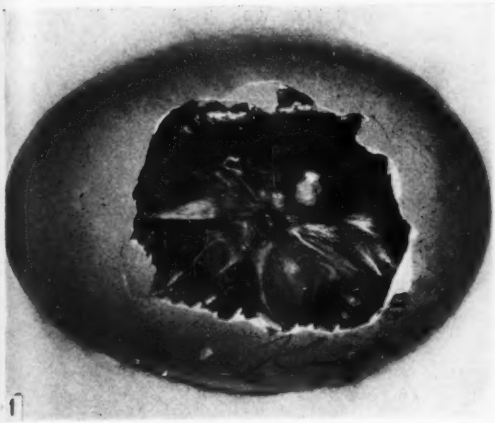
DESCRIPTION OF PLATES

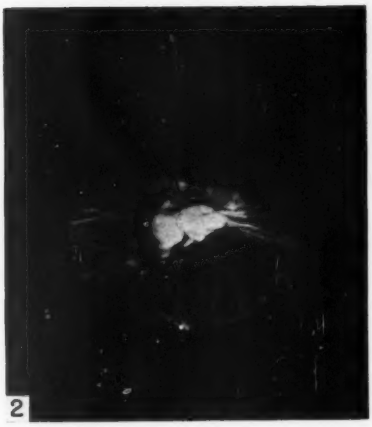
PLATE 20

FIG. 1. *In situ* view of the lesion produced by tsutsugamushi rickettsiae on the chorioallantoic membrane, 12th passage. Of note is the central ulcer which, in this case, is filled with a clear, viscid fluid. × 1.

FIG. 2. A reverse view (from the inner surface of the allantoic sac) of the lesion in Figure 1, showing the nodular mass. Large blood vessels supply the mass, and punctate "daughter colonies" are seen apart from the main nodule. × 1.

FIGS. 3-5. Sections through chorioallantoic nodules. Figure 3 shows an early stage, with centers of proliferation indicated by islands of darkly staining mesenchyme cells. In Figure 4 the centers of proliferation have spread and started to become confluent. At the left of the section is a large necrotic mass which has broken through the allantoic endoderm. The outer (chorionic) epithelium has started to slough off in the center of the section where the primary ulcer will form. Figure 5 shows advanced necrosis. A fringe of proliferating cells surrounds each necrotic area. At the lower part of the section, one necrotic mass has formed a channel into the allantoic sac. × 8.





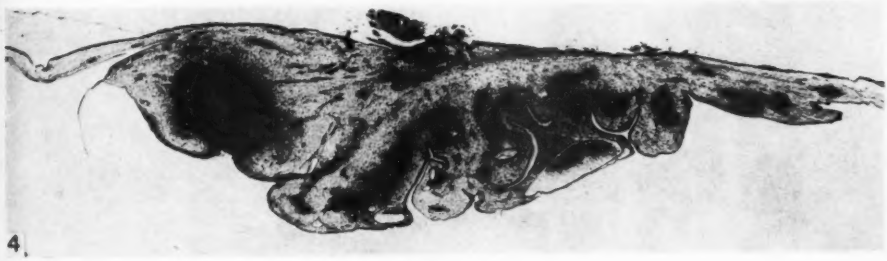


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Plate 21

Figs. 6 and 7. Portions of the allantoic endoderm, showing papillomatous-like thickening and multi-branched proliferation of the epithelium. × 120.

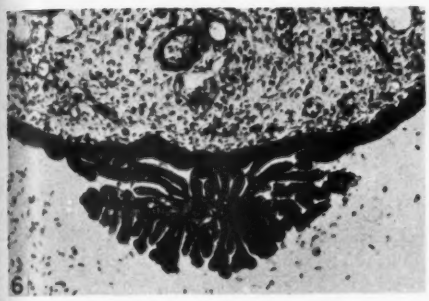
Fig. 8. A portion of the peripheral zone of a center of proliferation, showing the large number of "stem cells" which migrate into the mesenchyme and ingest rickettsiae. × 240.

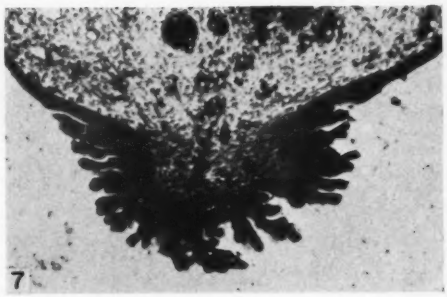
Fig. 9. Portion of a necrotic area, showing the surrounding ring of deeply staining mesenchyme in which the cells resemble fibroblasts, and in which rickettsiae are numerous. × 240.

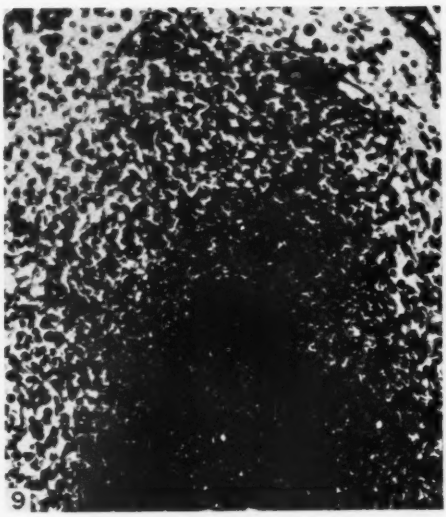
Fig. 10. Surface view of the chorionic epithelium, showing many rickettsiae. × 1660.

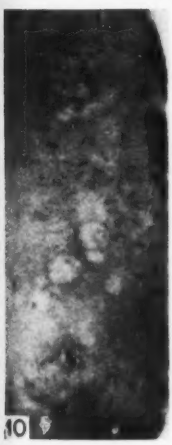
Figs. 11-13. Rickettsiae in the mesenchyme of the nodular mass. Figure 11 is from the fringe of an area of proliferation. Of note are the typical diplobacillary forms. × 1660.

Figures 3–13 are from U. S. Army Medical Museum negatives, numbers: 78866, 78868, 78867, 80420, 80419, 79150, 79151, 78924, 78870, 78869, and 78865, respectively.









Hamilton Rickettsiae of Tsutsugamushi Fever

LESIONS OF SKELETAL MUSCLES IN RHEUMATOID ARTHRITIS
NODULAR POLYMYOSITIS *

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Multiple nodular inflammatory lesions in peripheral nerves in cases of rheumatoid arthritis were reported for the first time in previous papers.[1,2] They were found in 3 of 5 cases. Identical findings observed in 5 more cases of this disease are to be reported in another paper. Since muscular atrophy, occasionally of rapidly progressive nature, is seen clinically in many cases of rheumatoid arthritis, an attempt was made to search for anatomopathological changes to which muscular atrophy could be related.

MATERIAL AND TECHNIC

Muscle in quantity is not easily obtainable during life. The patient is often reluctant to permit the removal of tissue for biopsy for scientific purposes alone without prospect of therapeutic benefit. Pieces of muscles taken for biopsy are usually small, rarely over 2 gm., and are taken at random from a large muscle of the arm or leg. In 4 cases of rheumatoid arthritis such specimens were taken from the gastrocnemius muscle; in 1 of these an additional specimen was taken from the deltoid muscle. In 2 cases specimens from the triceps muscle were taken for biopsy. In another case of rheumatoid arthritis a mid-thigh amputation of both legs was performed, because of immobility and marked deformity of the patient's lower extremities. The peripheral nerves of both legs were dissected. Some muscle tissue, adherent to these nerves, was obtained incidentally. In 2 cases pieces of muscle were taken at autopsy, in 1 from the pectoral muscle, in the second from the rectus abdominis, pectoral and iliopsoas muscles. The latter case was found at autopsy among our control material. Altogether, muscles from 9 cases of rheumatoid arthritis were examined.

Since pathological involvement of skeletal muscles in cases of systemic disease has been described frequently, a large number of controls was desirable. Up to the present time, 196 control cases, selected from routine autopsies and surgical operations without any previous knowledge of the history or diagnosis, have been examined. There were

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specimens from the recti abdominis, the pectorales, the iliopsoas, the gluteal muscles, the bicepses and tricepses.

The specimens of muscle were fixed in formalin, and blocks were cut transversely and longitudinally; after embedding in paraffin, sections were made 7 μ thick. Hematoxylin and eosin, cresyl violet, van Gieson's, and reticulin (Wilder) stains were used. Frozen sections could not be made because the small amount of muscle tissue obtained had to be embedded entirely in paraffin. Likewise there was no material available for fixation in alcohol, which excluded staining for glycogen. For similar reasons histopathological studies of striation and sarcomeres requiring special technics were not made.

No descriptions of similar findings are to be found in medical literature. The only description of pathological lesions resembling ours is in a paper by Curtis and Pollard.[3] They found increase in interstitial nuclei of the muscle fibers, small perivascular infiltrations, and atrophy of muscle fibers in specimens taken for biopsy from the calf muscles of 12 patients with rheumatoid arthritis. The purpose of their paper was to determine whether Felty's syndrome was a clinical entity or just an "expected complex of rheumatoid arthritis."

Report of Cases

Case 1

The patient (Eloise Hospital) was a white female, 54 years old, the mother of 11 children. For 19 years she had had rheumatoid arthritis involving nearly every joint. Roentgenograms revealed bony ankylosis of the left knee joint. The joint space of the right knee was lost on the lateral half, and on both sides there was some loss of joint space between the humerus and the ulna with partial bony ankylosis. The muscles of the lower extremities were markedly atrophic.

Morphological Description

The specimen was taken from the middle portion of the left gastrocnemius muscle and was 1.6 cm. long and 0.9 cm. thick in its largest transverse diameter. Grossly, it did not differ from a piece of normal muscle.

The pathological findings had to be separated into those which were inflammatory and those which were degenerative. At least five inflammatory lesions were found in each section. They were entirely separated from one another and consisted of nodular accumulations of lymphocytes and plasma cells. The plasma cells were more frequent in the peripheral portions of the nodules. The shapes of the nodules varied markedly. There were triangular, pyramidal, and spindle-shaped nodules (Fig. 1) located in the perimysium and the endomysium, separating single muscle fibers from each other. Occasionally

a small amount of adipose tissue was seen at one or both ends of the nodule (Fig. 2). In addition to the nodular accumulations, there were a few well demarcated foci composed of a smaller number of lymphocytes. There was no necrosis in the lymphocytic nodules, but occasionally there were seen a few epithelioid cells that had arisen from the cells of capillary endothelial linings or from the sarcolemma. Van Gieson's stain revealed an increase in collagenous connective tissue fibers while the reticulin stain failed to reveal an increase in reticulin fibers in the nodules.

The degenerative lesions of muscle fibers were well defined and were very irregularly distributed in close proximity to normal muscle fibers. A single degenerated fiber was never seen isolated between normal fibers; always three or more degenerated fibers were together. The degeneration of muscle fibers was marked by an increase of sarcolemmal nuclei, closely packed together; swelling or, more often, extreme shrinkage of fibers; a change of the staining reaction of the fibers from a bright red to pale red or pink (Fig. 2), and an increased coarseness in the cross striations with larger but regular interspaces between the striations. Occasionally a slightly swollen muscle fiber showed a transition to shrinkage, indicating a segmental degeneration. Extremely shrunken muscle fibers in cross section were not much larger, or were even smaller, than human red blood cells (Fig. 3). The nucleus of such a shrunken muscle fiber was located at the periphery. Occasionally an empty space like a vacuole was seen as the core of the shrunken muscle fiber, leaving at the periphery a pale, ring-shaped, membrane-like structure in which the nucleus was embedded. The nuclei were small, spindle-shaped or curved, and contained dark-staining chromatin. Occasionally a cross section through a shrunken muscle fiber revealed 8 to 15 small, dark nuclei in the center of the fiber.

In the walls of capillaries and larger blood vessels and inside perimysial nerve fiber bundles, as well as in neuromuscular spindles, no inflammatory nodules were seen. However, in perimysial locations adjacent to intramuscular nerve fiber bundles and to the adventitia of blood vessels nodular lymphocytic infiltrations were found (Figs. 4 and 5).

Case 2

The patient (Eloise Hospital), a white female, 48 years old, had had progressive rheumatoid arthritis for 19 years. She showed flexion deformities of the elbows, wrists, and fingers; complete ankylosis of the right wrist and partial ankylosis of the left; and nearly complete ankylosis of the left ankle and both knees with subluxation. Roentgenograms revealed a marked loss of joint space in the right interphalangeal joints, loss of joint space in the right elbow, and ankylosis of the right knee. The skin over the legs and on the face was glossy. There was severe muscular atrophy.

Morphological Description

The specimen for biopsy was taken from the middle portion of the left gastrocnemius muscle. It measured 2.4 cm. in length and was 0.6 cm. in its largest transverse diameter. There was no grossly visible change.

The microscopical findings again had to be separated into inflammatory and degenerative changes. The inflammatory lesions appeared in separated and remote groups. They were well circumscribed and occupied the perimysium and the endomysium of muscle bundles. Their shape was nodular but more irregular than that of the previously reported [1,2] perineuritic nodules; there were often small nests of cells connected with the nodule that in tongue-like fashion infiltrated farther into the endomysium between two muscle fibers.

The inflammatory cells were lymphocytes and plasma cells, the former being distributed very regularly throughout the nodules, the latter found in the periphery of the nodular accumulations. There was no definite inner zone of necrosis or of epithelioid cells. The nodules contained capillaries and increased collagenous connective tissue while the reticulin fiber network was scant.

Muscular degeneration was seen in very irregular distribution. Well preserved muscle fibers were seen in close proximity to severely atrophic muscle fibers (Fig. 6). There was swelling as well as shrinkage of the fibers, the swollen fibers showing discoloration, and usually a darker color. Cross striations were coarser and the interspaces larger. Instead of straight outer contours, the swollen muscle fibers showed wavy or irregularly distorted outlines. The shrunken muscle fibers were occasionally paler; nevertheless, the cross striations were often distinctly seen. The reduction in size was more than two-thirds of normal; in crosscut fibers the size of an individual fiber was often smaller than that of a red blood cell. The nuclei in these shrunken fibers were peripherally located; however, there was an occasional accumulation of 8 to 10 nuclei closely packed together. In some of the markedly shrunken muscle fibers only a nucleus and a membrane-like outer structure remained, and the inner core was represented by a vacuole. The perimysial connective tissue was increased in amount and showed more nuclei than are normally present. There were accumulations of nuclei even in the muscle fibers that did not exhibit degenerative changes. Occasionally an increased amount of adipose tissue could be seen between muscle fibers.

In the walls of capillaries and larger blood vessels and inside of perimysial nerve fiber bundles as well as in neuromuscular spindles no inflammatory nodules were seen. However, in the vicinity of two small

perimysial nerve fiber bundles, nodular infiltrations involving the perineurium and adjacent interstitial tissues were seen (Fig. 7).

Case 3

The patient (Harper Hospital) was a white male, 53 years of age, who was admitted to the hospital on May 29, 1944, complaining of painful and swollen joints. The condition had followed the removal of a tumor of the bladder 4 years previously. The joints were involved in the following order: feet, knees, hands, elbows, neck, and shoulders. Over a period of several months the joints had become swollen, stiff, and sore. The extremities were moist and cool. The condition was worse during cold or wet weather. The patient had become gradually weaker, and he noted that his muscles wasted as time went on. On two occasions he had had transitory "lumps" at his elbows. His muscles were always sore, especially those of his arms and legs. He had received fever therapy and a course of gold injections at another hospital, but he felt that his condition had not been improved.

The patient was a large man, somewhat pale, with marked deformity of all joints. There was fusiform swelling of the phalanges. The metacarpophalangeal and the carpometacarpal joints showed diffuse periarticular swelling; there was marked interosseous atrophy. The elbow joints could not be fully extended, and the arms could be raised only to an angle of 45° from his body. Motion of the feet and knees was limited. No nodules were felt. The heart was not enlarged, and no murmurs were present. The blood pressure was 140/85 mm. Hg. All reflexes were hyperactive. Sensation was normal everywhere except for some hyperesthesia of the feet.

During his stay at the hospital the patient had prostigmine, methylsalicylate, myochrysine, blood transfusions, massive doses of sodium salicylate, high vitamin-D medication, and physiotherapy. There was moderate improvement.

Laboratory Findings. Examination of the blood showed: Hemoglobin, 86 per cent; red blood cells, 4,460,000; white blood cells, 8,400, with a normal differential count. Several determinations of the sedimentation rate gave a maximum of 27 mm. and minimum of 21 mm. in 60 minutes. Blood nonprotein nitrogen was 36.6 mg.; blood calcium, 11.5 mg.; and blood phosphorus, 2.1 mg. per cent. A Kahn test of the blood was negative.

Radiological Examination. Soft tissue swelling was seen about the majority of the proximal interphalangeal joints with decrease in the joint spacing of all carpal bones and a generalized demineralization of all bones examined. No hypertrophic changes could be seen. There was roentgenographic evidence of arthritis, of a rheumatoid type, involving the left hand. Studies made of the right knee revealed thinning of the articular cartilages with considerable effusion into the bursae.

Morphological Description

Specimens were taken from the left gastrocnemius and deltoid muscles. The two pieces of tissue from the gastrocnemius muscle were 1.1 by 0.9 cm., and 0.4 by 0.2 cm. The piece from the deltoid muscle was approximately 1 by 1 cm. Grossly, there were no visible changes.

Except for an increase in sarcolemmal nuclei, no degenerative or inflammatory lesion was found in the gastrocnemius muscle. The specimen from the deltoid muscle showed two small inflammatory nodules of typical appearance, without atrophic lesions of the muscle fibers.

The inflammatory cells again were lymphocytes and a few plasma cells. Rarely, polymorphonuclear cells were seen.

In the walls of capillaries and larger blood vessels, in perimysial nerve fiber bundles, and in neuromuscular spindles no inflammatory nodules were seen.

Case 4

The patient (Harper Hospital) was a white male, 46 years old, whose chief complaint was painful, stiff, swollen joints. Six years before he had noticed occasional pain in the muscles of his left arm. This slowly progressed to the other arm, and then the knees became involved. He received treatment in many forms during this period of time, the last being 100,000 units of vitamin D daily. On this he improved slightly. At the time of entrance to the hospital he was totally disabled and could not walk. Occasionally, when the joint condition grew worse, hoarseness developed.

Past illnesses were septic sore throat in 1932; gonorrhea in 1935; malaria in 1935; and pneumonia in 1940. There was nothing remarkable in the family history, and there was nothing of importance in the physical examination except for the findings which concerned the joints. The heart was not enlarged. There were no murmurs and no arrhythmia. Blood pressure was 147/75 mm. Hg. The spleen was palpable. Examination of the joints revealed ulnar deviation with inability to extend the metacarpophalangeal joints. The elbows were slightly flexed as were the feet and knees.

Laboratory Findings. Examination of the blood showed: Hemoglobin, 80 per cent; red blood cells, 4,700,000; white blood cells, 8,750, with 60 per cent segmented forms, 34 per cent lymphocytes, and 6 per cent band forms. The sedimentation rate was 23 mm. in 60 minutes. Chemical studies showed nonprotein nitrogen of the blood to be 36.6 mg.; calcium, 11.1 mg., and sugar, 80 mg. per cent. A Kahn test upon the blood was negative.

Radiological Examination. Roentgenograms were made of the chest, teeth, paranasal sinuses, and gallbladder; no abnormal findings were recorded. Roentgenograms of the hands showed marked narrowing of the joint spaces with considerable demineralization of the bone, and there was some periarticular soft tissue swelling. The cartilaginous disks had almost entirely disappeared.

Clinical Diagnosis. The case was diagnosed as typical rheumatoid arthritis. The patient was discharged to his private physician with recommendations for the resumption of gold therapy, of which he had had inadequate amounts several years before.

Morphological Description

The specimen from the left triceps consisted of two pieces, one of oval shape measuring 0.5 and 0.6 cm., and a very small piece composed of but ten muscle fibers longitudinally cut. There was no grossly visible change in either of them. However, sections stained by hematoxylin and eosin showed grossly visible, dark blue dots separated from each other. The size and shape of these dots varied from round to elliptic. With a hand lens seven of these areas were counted in the larger pieces and two in the smaller. Their shape was irregular due to elongated processes of dark blue color extending from some of them.

In serial cuts these dark blue-staining spots extended through 10 to 15 consecutive sections. Since each section was 7 μ thick, the average size of such a nodule was 0.070 to 0.105 mm. in thickness, the maximum length being about 1 mm.

Microscopically, the inflammatory lesions were well circumscribed, although there were processes from the nodular mass infiltrating the perimysium and the endomysium (Fig. 8). These elongated processes were composed of the same cells as the nodules. The inflammatory cells were lymphocytes, many of them immature with vesicular nuclei. There were also rather abundant plasma cells in a more peripheral location, and mast cells outside the nodules. In larger nodules circumscribed accumulations of the epithelioid cells with nuclei poor in chromatin and definite nucleoli were seen (Figs. 9 and 10). These nuclei seemed to be related to capillary endothelial cells or sarcolemmal cells. They were grouped together, usually nearer to the periphery of the nodule. In sections stained with van Gieson's stain, the increase of collagenous fibers was marked, and the location of the inflammatory process in endomysium and perimysium could be ascertained. Many of the larger nodules showed a somewhat loose structure with spaces surrounding the lymphocytes and plasma cells. No stroma was recognized in these spaces; a reticulin stain revealed scanty reticulin fibrils. In one elongated nodule the accumulation of inflammatory cells was interrupted by a crosscut capillary, the walls of which were not infiltrated by inflammatory cells. Aside from larger nodules, small foci of lymphocytic infiltration were seen more widely spread.

Of special interest were small inflammatory foci where a muscle fiber was surrounded by endomysially located inflammatory cells, lymphocytes, and plasma cells. Here a beginning degenerative alteration of the muscle fiber was seen (Fig. 11). There was an occasional peculiar arrangement of small vacuoles in longitudinal rows parallel with the longitudinal striations of the fiber, which then did not show any cross striation (Fig. 12). These muscle fibers were much paler than the normal ones in their vicinity and toward their peripheral portions showed increased large nuclei poor in chromatin. These nuclei regularly contained two nucleoli, were elliptic or ovoid, and showed a distinctly outlined nuclear membrane; occasionally the nuclei were distributed in rows close together at the periphery of the muscle fiber (Figs. 11 and 13). Rarely, one large elongated nuclear mass containing dark-staining chromatin was seen instead of the numerous pale nuclei described before (Fig. 14). In the pale gray-staining sarcoplasm of these degenerating muscle fibers peculiarly pale-staining granular

dots of minimal size were seen. Coarser cross striation, beginning longitudinal fibrillation, and beginning vacuolization around hyperplastic muscular nuclei were seen in some of the muscle fibers (Figs. 15 and 16). Most of the muscle fibers, however, did not show beginning or advanced degeneration although there was occasional swelling.

The epimysium showed no signs of inflammation, nor did the walls of capillaries and of larger blood vessels, the perineurium of intramuscular nerves, or the neuromuscular spindles.

Case 5

The patient, a white male, 61 years old, was admitted (Harper Hospital) on August 4, 1944, because of pain in his joints. He had suffered from rheumatoid arthritis for 4 months, being troubled with pain that seemed to originate at the back of his neck. His attending physician believed that the discomfort on flexing his neck was due to "abscessed" teeth. These were removed. Following the extraction, however, the pain spread to the joints of his upper extremities, affecting chiefly the elbows and fingers. During the month prior to his admission he developed pain in both knees.

There was nothing remarkable on physical examination except a slight increase in the transverse diameter of the heart; a blowing systolic murmur was heard at the apex and transmitted toward the base, P_2 being slightly accentuated. Blood pressure was 120/60 mm. Hg. The margin of the liver was palpable; the spleen was not palpable. Except for hyperactivity in his reflexes there were no abnormal neurological findings. There was marked pain on flexion of the knee joints and slight muscular atrophy above and below the joints. In the upper extremities there was slight fusiform swelling of the first phalangeal joints and some thickening of the distal joints of his fingers. The amount of pain and stiffness was variable; at one time he was unable to lift his arm to his head. There were slight elevations of temperature during his stay in the hospital.

The patient was placed on myochrysine therapy with marked improvement.

Laboratory Findings. Examination of the blood showed: Hemoglobin, 76 per cent; red blood cells, 3,910,000; white blood cells, 9,050, with a differential count of 6 per cent band forms, 68 per cent segmented forms, 24 per cent lymphocytes, and 2 per cent eosinophils. Sedimentation rate was 20 mm. in 60 minutes. Nonprotein nitrogen of the blood was 48.6 mg. per cent. The Kahn test was negative. There was a slight trace of albumin in the urine and occasional fine granular casts.

Radiological Examination. Studies of both hands revealed some periarticular swelling of soft tissues with a very slight demineralization of the bones. Although there was no disturbance in joint spacing except slight thinning of the cartilages of the joints, particularly of the metacarpophalangeal joints, the film study showed arthritis of an early atrophic type.

Morphological Description

Two pieces were taken from the left gastrocnemius muscle. One was 0.9 and 0.4 cm. in longitudinal and transverse diameters respectively; the other was 0.7 by 0.5 cm. Grossly, there was no visible change. Microscopically, there were again lesions of two distinct types, inflammatory and degenerative. The inflammatory lesions were of a

definitely nodular type. In the entire section five nodules were seen, four in the perimysium and endomysium, and one in the epimysium. The nodules seen in crosscut muscle fiber bundles were endomysial; the muscle fibers themselves were not invaded by inflammatory cells (Fig. 17). As before, the inflammatory cells were lymphocytes and plasma cells. The nodules were small, much smaller than in the previous case; they did not contain epithelioid cells and apparently represented earlier stages of muscle involvement than in the previous case. Nevertheless, the collagenous connective tissue was increased in and around the nodules. The epimysial nodule had developed between blood vessels, but there was no involvement of the vascular walls. The inflammatory cells were spread between the adventitial layers of the blood vessels, and no inflammation was present in the adventitia, media, or intima of the vascular walls.

In spite of the smallness of the inflammatory nodules, scanty but definite signs of degeneration of muscle fibers were seen. The degenerating muscle fibers were located between normal muscle fibers, the ratio of normal to degenerating muscle fibers being 20 to 1. There was a definite color change of the degenerating fibers; some exhibited a more bluish tinge, others a bright, peculiarly refractile, yellowish color (in hematoxylin and eosin stain). The discolored fibers, when longitudinally cut, showed a marked longitudinal fibrillation. The degenerated fibers showed somewhat irregular lines of cross striations and coarser interspaces between the transverse striae. Most of the bluish muscle fibers showed an increase of large, pale nuclei with one or two large nucleoli; the nuclei were sometimes arranged in longitudinal rows. In these fibers vacuolization at the periphery of the muscle fiber or around the large nuclei was occasionally seen (Fig. 18). Again, this early lesion seemed to be segmental, occupying a short longitudinal portion of a single fiber. Neuromuscular spindles were free of inflammation.

Case 6

The patient, a white female, 29 years old, was admitted (Harper Hospital) on August 14, 1944, having been in good health until 13 months previously when she had begun to suffer with rheumatoid arthritis. Three weeks after the birth of her first child she had felt pains and stiffness in the muscles and joints. At the outset, the symptoms came and went without any evidence of swelling, but a short time later she noticed slight swelling, redness, and tenderness. At that time an "abscessed" tooth was extracted. There was no history of any other previous acute infection or chronic illness. The patient had been carefully examined in two other medical clinics: in one no diagnosis was made; in the other, because of an increased sedimentation rate, she was told that the possibility of rheumatoid arthritis had to be considered. Roentgenograms of the joints at each clinic revealed no changes suggestive of rheumatoid arthritis.

At the first examination the patient complained as much of pain in the muscles, particularly in the deltoid muscles, as of pain in the joints. She was worse at night, and when awakening found it difficult to move because of pain and stiffness.

The skin was somewhat glossy, delicate, moist, and smooth. The heart sounds were clear and of good quality. There was a slight spindle-shaped swelling of the fingers of each hand, and a very slight interosseous atrophy of the dorsum of each hand. The right great toe was stiff, but no enlargement could be determined. The reflexes of the extensor muscles of the forearm, the biceps, and the patellars were hyperactive. No clonus was elicited. During the 9 days that the patient was in the hospital her temperature rose to 101° F. on two occasions and promptly subsided. The pulse rate varied from 80 to 110. Myochrysine therapy was begun during hospitalization.

The patient presented the clinical features of early rheumatoid arthritis.

Laboratory Findings. Examination of the blood gave the following results: Hemoglobin, 78 per cent; red blood cells, 3,910,000; white blood cells, 4,150, with a normal differential count. The sedimentation rate was 24 mm. in 60 minutes. A Kahn test upon the blood was negative. Examination of the urine gave only negative findings.

Radiological Examination. Examined in dorsopalmar projections, the hands showed no evidence of abnormality. When these roentgenograms were examined 1 month later, one roentgenologist thought that some slight narrowing of the joint spaces was present, but no destructive process was discernible.

Morphological Description

Two small pieces were obtained for biopsy from the left triceps muscle. The larger was 0.4 cm. in longitudinal and transverse diameters; the smaller was a pyramidal-shaped piece with a 0.3 cm. longitudinal axis and a 0.4 cm. base. Microscopically the inflammatory nodules were smaller than those seen in the three previous cases; their morphological appearance, however, was identical. Individual degenerating muscle fibers were seen of the same type as those in case 5, but they were fewer.

Case 7

The patient (Eloise Hospital) was a white female, 27 years of age, who was admitted in January, 1942, because of swollen and painful joints. At the age of 11 she had had swelling and redness of the left knee that disappeared after rest in bed; the pain did not migrate. The patient did not recall that she had had any fever. She had been in good health until about 6 years prior to admission, when pain and stiffness appeared in the right knee, both shoulders, and in the right temporomandibular joint. Later she had swelling and severe pain in the knees. An impacted wisdom tooth was extracted, the right knee joint was aspirated, and she received vaccine and physiotherapy, but no relief was forthcoming. She was able to get about on crutches. Following the delivery of a full-term child 3 years before admission to the hospital, her condition became worse. Swelling and tenderness extended to other joints of her body, notably to the fingers of both hands. Two years before admission both legs and the left elbow joint were put in casts. No improvement followed attempts to straighten the legs and to manipulate the joints.

On admission, the skin was pale, cool, thin, and shiny, especially over the extremities. The heart was not enlarged nor were there any murmurs. All joints

of the body were involved, with marked deformity, limitation of motion, and fixation of some of them, especially the knees and ankles.

Laboratory Findings. The blood showed: Hemoglobin, 10 gm.; red blood cells, 4,200,000; white blood cells, 4,400, with 40 per cent filamented forms, 4 per cent nonfilamented forms, 39 per cent lymphocytes, 6 per cent large mononuclear cells, and 11 per cent eosinophils. The sedimentation rate was 20 mm. in 60 minutes. A Kahn test was negative. Urinary examination showed no abnormality, and the electrocardiographic tracings were considered normal.

Radiological Examination. The fingers and wrists showed osteoporosis and sharply outlined joint margins. Similar changes were seen in both elbows, knees, and feet. The right knee showed, in addition, a fused patella and apparent fusion of the tibia and fibula. In both ankles there was tibial-tarsal fusion. The proximal phalanges of both feet were dislocated; marked hallux valgus was present.

Clinical Diagnosis and Course. The diagnosis was advanced rheumatoid arthritis, trophoneurotic disturbances, and hypochromic microcytic anemia.

Treatment consisted of blood transfusions, sedatives, and physiotherapy. Kirschner wires were inserted into both tibiae and traction applied. Two months later osteotomy of the right knee was performed and a plaster dressing at 10° flexion applied. One month later roentgenograms showed fusion of the tibia and femur, and union of patella with the right femur. The ankylosis and deformity of the lower extremities gave the patient constant pain and almost total immobility. She was able to use her upper extremities somewhat. The patient and her husband demanded consideration of the fact that she could be more easily handled at home if the useless legs were removed. First the right leg was removed at the mid-thigh, and 3 weeks later the left leg at the mid-thigh. She made an uneventful recovery and was discharged.

At the time of amputation our interest was focused on the investigation of peripheral nerves. By chance, some muscle tissue adherent to the left tibial nerve and another piece of muscle adherent to the left superficial peroneal nerve were removed. The nerves showed typical nodulous rheumarthritic perineuritis.

Morphological Description

In the muscle tissue adherent to the left tibial nerve two nodules were seen. One was the largest in this series of cases. It was seen grossly in stained sections as a deep-blue dot, 1 mm. in transverse diameter and 1.1 mm. in longitudinal axis. The other nodule was small and was seen only microscopically. The muscle tissues in the vicinity of the left superficial peroneal nerve showed three small nodules.

Again, inflammatory and degenerative lesions were distinguishable. The large nodule showed a massive accumulation of lymphocytes and plasma cells, the latter at the periphery. Epithelioid cells were not conspicuous; there were, however, remnants of degenerating muscle fibers in the center of the nodule as well as at its periphery (Figs. 19 and 20). Not far from the nodule, in the perimysium, an artery was seen with nodular inflammation of its wall, mostly in the media and adventitia (Figs. 21 and 22). A marked increase in collagenous connective tissue fibers was seen in the nodule and in its surroundings, with no increase in reticulin fibers. In the vicinity of the nodule the muscle fibers showed severe degeneration, a lack of cross striation, vacuolization, dis-

tortion of the straight longitudinal form with wave-like outer contours, and an increase in large nuclei that were poor in chromatin and contained one or two prominent nucleoli. Occasionally the muscle fibers were broken up into smaller, irregular fragments containing these large nuclei.

In the muscle in the vicinity of the left superficial peroneal nerve one perineurial nodular accumulation of lymphocytes and plasma cells was seen; the perineurium of a small intramuscular nerve fiber bundle was completely infiltrated with lymphocytes and plasma cells (Fig. 23). The other two nodules were endomysial without relationship to nerves or blood vessels. There was a severe shrinkage of muscle fibers in isolated nests as described in previous cases.

Case 8

The patient was a white male, 55 years old. When he entered Eloise Hospital in August, 1941, complaining of painful, swollen joints, he had been suffering with rheumatoid arthritis for more than 11 years. Eleven years before he had noted pain in the left hand and elbow and some swelling and reddening of the joints. Progressively, over a period of several months, the shoulders, knees, hands, and feet became involved. Treatment was of no avail, and gradually his hands and feet became deformed. Numbness and tingling of the toes occurred at various times, and cramping of the calf muscles was an occasional symptom. Some shortness of breath was experienced on exertion. For the past 3 years slight swelling of the ankles had been noticed.

On admission his temperature was 98° F.; the pulse, 90; and respiration, 20. The eyegrounds showed some arterial tortuosity; the tonsils were atrophic; and the mouth was edentulous. Signs of fluid were present in the left pleural cavity, and a few moist râles were present at each base. The heart dulness merged with the dulness at the left base. There were no murmurs and no arrhythmia, although the aortic second sound was slightly accentuated. Blood pressure was 158/90 mm. Hg. All joints of the body were involved. There was marked limitation of motion of the larger joints. The fingers were flexed and rigid. Atrophy of all muscles of the body was apparent, especially those of the hands, arms, legs, and thighs. The feet and legs were cool, the right cooler than the left. Pulsation in the dorsalis pedis was less noticeable in the right foot than in the left. The reflexes were all markedly exaggerated. Clonus, muscle spasm, and fibrillation were not present. Subcutaneous nodules were present at each elbow. A nodule was excised for biopsy.

In September, 1941, cardiac decompensation developed, but the patient recovered temporarily following appropriate therapy. In August, 1942, the right great toe became infected, and gangrene of the foot followed. Cardiac decompensation returned. A right mid-thigh amputation was performed but the patient's condition did not improve and he died of cardiac failure on October 10, 1942, 6 weeks following amputation.

Laboratory Findings. Examination of the blood showed: Hemoglobin, 9.5 gm.; red blood cells, 3,780,000; white blood cells, 9,600; blood sedimentation on admission, 20 mm. in 60 minutes. A Kahn test was negative. Examination of the urine showed albuminuria of mild degree, with a few hyaline and finely granular casts; the average specific gravity was 1,020. Electrocardiograms revealed severe myocardial damage.

Radiological Examination. There was marked contracture deformity of the left hand. All proximal phalanges were dislocated in relation to the heads of the metacarpals. The joints of the wrists were narrowed. The joint spaces of the left elbow were narrowed and the margins sclerotic and irregular. Both patellae were fixed and the joint spaces of the knees were narrowed with sclerotic margins. The same changes were present in the ankles.

Clinical Diagnosis. Advanced rheumatoid arthritis, subcutaneous nodules, generalized arteriosclerosis, arteriosclerotic heart disease, myocardial failure, bilateral hydrothorax, gangrene of the leg, and peripheral arteriosclerotic disease.

Morphological Description

The findings at autopsy were bilateral purulent empyema, bilateral chronic adhesive pleuritis, hypertensive and atherosclerotic heart disease, chronic fibrinous pericarditis with obliteration of the pericardial sac, atherosclerosis, and arteriolar sclerosis of both kidneys.

At the autopsy, three pieces of tissue were taken from the left pectoralis major muscle. In each of the three there was nodular lymphocytic inflammation, with usually three to five nodules in each section. They were located in the perimysium, more rarely in the endomysium. Small, elongated, spindle-shaped, circumscribed, inflammatory foci were seen in the endomysium (Fig. 24). An increase of collagenous connective tissue fibers and a lack of reticulin fibril production were apparent as usual. The perimysial nodules were often seen in the vicinity of blood vessels periadventitially, but never adventitially (Fig. 25). There was an occasional nodule in the perineurium of an intramuscular nerve bundle. The muscle fibers showed irregularly distributed swelling and a loss of cross striation. There were a few severely shrunken muscle fibers, usually in bundles of two or three between normal fibers. The shrinkage was seen in both crosscut and longitudinally cut fibers.

In one of the pieces of muscle tissues there was arteriolar sclerosis with atherosclerosis in the larger arteries.

Case 9

This case was found among our 196 control cases. For controls, specimens were removed routinely at autopsy from the pectoral, iliopsoas, and rectus abdominis muscles. They were fixed in formalin and brought to the laboratory without any clinical or post-mortem data.

The patient was a white male, 55 years of age, who had died in the admitting room of the hospital after 1 week of illness. No other data were available. At autopsy left lobar pneumonia (pneumococcus, type VII), fibrinous pleuritis, hyaline perisplenitis, and perihepatitis were found. In addition the patient showed ulnar deviation of both hands and bilateral hallux valgus with lateral deviation of the other toes of both feet. He had multiple subcutaneous nodules; one at the knee joint was saved for microscopical examination. The diagnosis of arthritis had been made post mortem.

Morphological Description

Pieces from the iliopsoas and pectoralis muscles showed no conspicuous lesions; however, in the piece from the rectus muscle two distinct inflammatory nodules were found. They were located in the perimysial connective tissue near a nerve and an arteriole. There was no infiltration of the endoneurium of this nerve and no perivascular arrangement. The inflammatory cells were lymphocytes and plasma cells; van Gieson's and reticulin stains revealed the same characteristics seen in the nodules in the muscles of the previously described cases. Definite degenerative changes of the muscle fibers were not found.

The subcutaneous nodule was composed of a large central zone of necrosis in which cells and connective tissue had completely disappeared, being replaced by necrotic structureless masses. Peripheral to the necrotic zone, rows of radially arranged proliferating cells of fibroblastic origin were seen. The necrotic center was sharply demarcated by the palisading arrangement of these fibroblasts. In the peripheral zones of the nodule marked infiltration with lymphocytes and plasma cells was present, usually in perivascular arrangement. There were three smaller nodules of miliary size in the vicinity of the large nodule. In these smaller nodules the central necrotic zone was in the beginning stage, showing numerous fibroblasts resembling epithelioid cells, and both fine and thick, bright-staining, collagenous connective tissue bundles in a somewhat radiating arrangement.

Discussion

The muscular lesions reported herein were of an inflammatory nature. There were also degenerative changes in muscle fibers. The inflammation was of a nodular type, lymphocytes and plasma cells were abundant, mast cells occasional, and polymorphonuclear cells and eosinophils rare or absent. The size of the nodules varied from those seen grossly in stained sections to very small ones. The shape was round or oval, triangular or spindle-shaped, often elongated, with processes of inflammatory cells infiltrating the endomysium between two or more muscle fibers. There were also smaller foci composed of twenty or less lymphocytes. The inflammatory nodules were located in the endomysium and the perimysium, rarely in the epimysium. In the larger nodules cells of an epithelioid type were seen, usually not in the center of the nodule but at the periphery. The origin of these cells could not be established definitely. They were derived either from capillary endothelial cells or from muscular nuclei, for there was a

definite resemblance between these nuclei and those of degenerating muscle fibers. The inflammatory nodules were found in each of the nine cases of rheumatoid arthritis examined. Striking features of the nodules were an increase in bundles of collagenous connective tissue fibers and a lack of reticulin network. Even in larger nodules a loose structure was evident with spaces around the inflammatory lymphocytes and plasma cells.

There were various stages of degeneration of muscle fibers. In late cases of rheumatoid arthritis, such as our cases 1 and 2, both of 19 years' duration, the degenerative changes were advanced and were represented by a marked atrophy, fatty metamorphosis, and severe distortion of the outer contours of the fibers. The early stages of muscle degeneration were seen in our cases 4, 5, and 6 (of 6 years', of 4 months', and of 13 months' duration respectively). They were distinct from the late stages, in which shrinkage was the most prominent feature. In the early stages of involvement there was a change in color to a bluish tinge or, more rarely, to a peculiar bright yellow. The cross striations were not straight but somewhat wavy, and the interspaces between them were coarser than normally. There were pale muscle fibers and peculiar vacuolization of these fibers. The bluish discoloration was somewhat similar to that reported in the basophilic granular degeneration of muscle fibers in trichinosis. The muscular nuclei were more numerous, and an alteration of position and shape was seen. Instead of being just beneath the sarcolemmal sheath the nuclei were located more toward the interior of the fibers or in the central longitudinal axis. The nuclear shape changed from rod-like to oval or round, and the nuclei became twice as large as normal. The perinuclear cytoplasm was not distinct; there was, however, perinuclear vacuolization. Densification of perinuclear sarcoplasm was seen also, the sarcoplasm exhibiting a darker color and a distinctly granular appearance. The nuclear chromatin was poor; the nucleolus was large, and there were often two nucleoli, which took the acid stain. Amitotic divisions and incomplete segmentation between nuclei were also found. Rarely an elongated giant nucleus was observed, its longitudinal axis being three times as long as the axis of one of the swollen nuclei. The proliferated nuclei appeared in longitudinal rows parallel with the longitudinal axis of the muscle fiber. Such rows contained as many as twenty nuclei.

In three cases the perineurium of intramuscular nerves showed nodular inflammation, and occasionally periadventitial and mural nodular inflammation of blood vessel walls was seen in the perimysium and the

epimysium. However, the early stages of the disease showed nodular inflammation in the endomysium and the perimysium without relationship to intramuscular nerve fiber bundles, blood vessels, or neuromuscular spindles.

Classification

The anatomopathological lesion in the muscular tissues must be designated as nodular polymyositis with secondary muscular atrophy. The name, polymyositis, has been selected because of the multiplicity of the inflammatory lesions and their wide distribution over the entire skeletal muscular system (gastrocnemius, other unidentified muscles of legs (case 7), deltoid, triceps, pectoral, and rectus abdominis muscles). The term "nodular" has been chosen in spite of occasional, more diffuse, inflammatory changes in the vicinity of the nodules because in most of the sections the muscular nodules appear as well demarcated lesions.

Perimysium and endomysium as well as muscle fiber and nuclei are of mesodermal origin. Since connective tissue, in general, and perimysium and endomysium, in particular, have a low grade of differentiation, the histological response to various infections and to other stimuli is limited. Among the infectious diseases a number of characteristic histological reactions of connective tissue can be recognized, for instance: tuberculous caseous necrosis, gummatous reticular fibrosis, fibrinoid necrosis with cellular palisading in subcutaneous nodules of rheumatic fever and rheumatoid arthritis, and Aschoff bodies in the myocardium. All of these more or less specific granulomatous reactions are characterized by the appearance of circumscribed nodules of varying sizes, composed of special tissue elements in special arrangements. Hence these processes are distinguishable from diffuse inflammatory infiltrations. The character of the infiltrating inflammatory cells is another factor determining the classification of inflammatory changes. Granulomas, in general, exhibit the subacute or chronic picture of lymphocytic cellular infiltration. Besides the characteristic appearance in nodules, there is often a lymphocytic accumulation in ring-like fashion at the periphery of the lesion. This lymphocytic ring is typical in nodular perineuritis of rheumatoid arthritis; the inner zone is occupied by epithelioid cells or fibroblasts. In the small nodular granulomas in the muscles of our patients, lymphocytes and plasma cells were evenly distributed throughout the entire granuloma; epithelioid cells were seen only in the larger nodules. Lymphocytes and plasma cells predominated; eosinophils and polymorphonuclear cells were rare. In rheumarthritic perineuritis the nerve fibers themselves did not ap-

pear to be damaged while in rheumatoid polymyositis a severe degeneration of muscle fibers accompanied the inflammatory process.

The muscular lesion found in our cases of rheumatoid arthritis may be classified as nodular polymyositis.

Nodulous rheumarthritic perineuritis as reported in previous papers [1,2] and nodular polymyositis described herein are essential findings in rheumatoid arthritis. Both lesions are of an inflammatory nature, are found in perineurial, and in perimysial and endomysial locations, and are alike in their histological patterns. Therefore, the findings in peripheral nerves and muscles may be classified in a single phrase as nodular neuromyositis of rheumatoid arthritis.

Specificity of Muscular Lesions

The histological observations reported herein are thought to be of specific character.

1. Nodular Appearance and Distribution. One of the main characteristics of the muscular lesions is their nodular appearance. Our material was not extensive enough to supply data on the distribution and preferred location of these nodular lesions in the entire mass of one skeletal muscle, to say nothing about the distribution in the entire skeletal muscular system or about the predilection of one or more individual muscles. Up to the present time the muscles examined are the gastrocnemius, deltoid, triceps, rectus abdominis, and pectoralis major. Our controls were the musculus pectoralis major, m. rectus, and m. iliopsoas. Our control material of 196 cases was not sufficient for a completely adequate comparison. Further, our control muscles showed other pathological lesions (atherosclerotic and arteriolar lesions, cartilaginous metaplasia in perimysial adipose tissue, post-traumatic abscess formation, trichinosis) in addition to those found in the cases of rheumatoid arthritis. Only in one control case were small lymphocytic infiltrations found in the endomysium and perimysium; these, however, were without secondary muscular atrophy. This was in a case of subacute bacterial endocarditis superimposed on old rheumatic heart disease. Another control case (reported herein as case 9) proved to be a case of rheumatoid arthritis.

Characteristic of the muscular change was the irregular distribution of the inflammatory lesions throughout the muscle tissues; in one section one to five nodules were seen, and the remaining perimysial spaces were without inflammatory lesions, or the lesions were very mild compared with the nodular accumulations. In serial sections these nodular inflammatory lesions did not extend through more than 10 to 15 sections, each 7 μ in thickness. Occasionally nodules were found in the

perineurium of small intramuscular nerves and in the walls of small arteries and veins. The intramural vascular inflammation was less often seen than the periadventitial arrangement. The neuromuscular spindles were never involved. An interesting histological detail was noted; namely, the increase of collagenous connective tissue and the decrease of reticulin fibers in the nodules.

2. Constancy of Occurrence and Uniformity in Appearance. Differential Diagnosis. Of a total of 10 muscle specimens from 9 cases, 7 were obtained for biopsy (2 from one case), and 3 through amputation of legs or at autopsy. Nine of these specimens showed definite and identical pathological lesions. The muscle tissues of each case were positive. In case 3 the specimen from the deltoid muscle was positive but not that from the gastrocnemius muscle. Although the amount of muscle tissue examined in each individual case was very small compared with the total quantity in the body, the high incidence of positive findings in various muscles is an indication of the wide distribution of nodular polymyositis throughout the skeletal muscular system in rheumatoid arthritis.*

Identical findings of nodular polymyositis in all 9 cases suggest the specific nature of this lesion. The control muscles did not show any lesions comparable to those found in rheumatoid arthritis. The controls were muscles taken from routine autopsies (196 cases) and 7 cases in which pathological lesions in muscles could be expected; namely, 1 case each of congenital myatonia, of myasthenia gravis, of amyotrophic lateral sclerosis, of progressive muscular dystrophy, of dermatomyositis, of lupus erythematodes, and of trichinosis. In addition to the previously mentioned pathological findings in our control material from routine autopsies, muscular degeneration without inflammatory lesions was found in cases of amyotrophic lateral sclerosis, of congenital myatonia, and of progressive muscular dystrophy. The inflammatory lesions in dermatomyositis and trichinosis were easily distinguished from those seen in the muscles of cases of rheumatoid arthritis. In dermatomyositis the inflammatory changes were diffusely spread and did not occur in nodular fashion as they did in the muscles of rheumatoid arthritis. In trichinosis, inflammation was close to encysted trichinae, with a definite capsule and numerous eosinophilic cells. There are a number of other diseases with definite muscular involvement; for example, glanders and Chagas' disease, that could not be examined for lack of material. On the other hand, in at least 7 of

* Five additional muscle specimens from 5 other cases of typical rheumatoid arthritis have shown identical lesions.

the 9 cases of rheumatoid arthritis from which muscle tissues were taken, there was not the slightest possibility of any other disease; they were typical cases of rheumatoid arthritis without any complication.

3. Morphological Similarity to Other Lesions Seen in Rheumatoid Arthritis. There is a striking resemblance between the muscular lesions in rheumatoid arthritis and those in peripheral nerves; the nodular arrangement is seen in muscles and peripheral nerves alike. Both lesions have the same type of cellular infiltration, lymphocytes and plasma cells. The location in the perimysium corresponds to that in the perineurium. The nodular perineurial inflammation in small intramuscular nerves is identical with that seen in nodulous rheumarthritic perineuritis. The general pattern of the lesions found in the muscles is quite similar also to that of lesions in the synovia, in subcutaneous nodules, and in scleromalacia perforans. An inflammatory nodular character prevails everywhere. In one of our cases (case 9) multiple subcutaneous nodules were present, one of which was examined microscopically. It presented the typical histological picture of a subcutaneous nodule in rheumatoid arthritis.

4. Histological Comparison with "Fibrositis." In favor of the specificity of the muscular lesions in rheumatoid arthritis is the entirely different histological picture in "fibrositis," which occasionally is characterized by the appearance of gross fibrous nodules. Hench,[4] declaring the fibrositic nodules as the "signposts of the disease," emphasized the disappointing results following biopsy. He found little or no histological abnormality. Other observers (Collins,[5] Slocumb,[6] Buckley[7]) agreed that the structural changes in fibrositic lesions are meager. The pathological findings in "fibrositis" as first described by Stockman;[8] namely, numerous fibroblasts, serous or serofibrinous exudate, and thickening of walls of the small blood vessels and nerve sheaths, were considered by Collins as unimportant. Collins emphasized the absence of cellular infiltration as the only important fact of Stockman's investigation. At any rate, there is not the slightest similarity between the muscular lesions in rheumatoid arthritis and the findings in "fibrositis," a strong point favoring the specificity of polymyositis in rheumatoid arthritis.

5. Comparison of the Skeletal Muscular Lesions in Rheumatoid Arthritis with Those in Rheumatic Fever. Klinge[9, 10] showed photomicrographs of granulomas in the musculus constrictor pharyngis and in the diaphragm of a patient with a second attack of rheumatic fever and rheumatic endocarditis. He considered these rheumatic nodules in the striated muscles more or less characteristic. They were often found

in the vicinity of joints but were also independent of the joints, for example, in the diaphragm and the pharynx. Graeff [11] also found typical granulomas in the voluntary muscles in rheumatic fever.

An interesting finding, heretofore not reported, is the involvement of the blood vessel walls in the muscles of rheumatoid arthritis. Definite nodular arteritis and periarteritis could be seen in small muscular branches in some of our cases. Von Glahn and Pappenheimer [12] described specific lesions of the small peripheral arterioles and capillaries in 10 of 47 cases of rheumatic cardiac disease, but skeletal muscles were not included in their routine autopsy material. As Collins [5] stated, polyarteritis has been observed frequently in rheumatic fever, but in rheumatoid arthritis the vascular lesions seem to be confined to the joints or subcutaneous nodes. This statement is no longer valid.

Pathogenetic Relationship between the Various Local Manifestations in Rheumatoid Arthritis

Rheumatoid arthritis is a systemic disease with many local manifestations such as inflammation in the synovia, in joint capsules, and in periarticular soft tissues; subcutaneous nodules; and inflammatory nodules in peripheral nerves and skeletal muscles.

1. Relationship between Lesions of Joints, Periarticular Lesions, and Lesions in the Muscles. In a previous paper,[2] emphasis was laid on the fact that the perineuritic nodules were not in contact with synovial or periarticular lesions. This independence applies also to the muscular changes, since specimens were taken for biopsy from gastrocnemius or triceps muscles far from any joint or periarticular tissues. In one of our cases the rectus abdominis showed nodular inflammation. There was no relationship of the muscular lesions to subcutaneous nodules, since, with two exceptions (cases 8 and 9), no subcutaneous nodules were present in the cases from which the specimens were taken.

2. Relationship between Perineuritis and Polymyositis. Theoretically it might be assumed that the muscular lesion results from damage done to the peripheral nerves in nodulous perineuritis. In our previous paper [2] reasons were presented why clinically manifested muscular atrophy seemed to us not at all related to perineuritic lesions. Also we were not able to disclose degenerative changes in axis cylinders or myelin sheaths of peripheral nerve fibers. Hence there is little foundation for the assumption that muscular lesions are secondary to perineuritis. Furthermore, the irregular distribution and the nodular type of lesion speak in favor of the autochthonous origin of the muscular lesions. There is often a perineurial inflammation of small intra-

muscular nerves, to be sure, but serial sections show this change to be discontinuous. The process certainly does not ascend from the intramuscular nerve twigs into the larger bundles of the nerve trunk. It also does not descend from a perineuritic lesion in the peripheral trunk into smaller intramuscular ramifications of nerve fiber bundles. There is no pathogenetic interdependence between the lesions in the trunks of peripheral nerves and the muscular lesions.

3. Relationship between Polymyositis and Muscle Degeneration. While perineuritis did not produce grossly or microscopically visible damage in adjacent nerve fibers, there seemed to be a close relationship between polymyositis and muscular degeneration. The myositis is certainly not a consequence of a primary muscle degeneration, for, in our control material of purely degenerative diseases affecting the skeletal muscular system, marked inflammatory changes were absent. In degenerative lesions of the muscle Hassin [13] has observed scanty infiltrations in the perimysium, but these are not nodular as they are in polymyositis of rheumatoid arthritis, and they are accompanied neither by perineuritis of the small intramuscular nerve fiber bundles nor by arteritis of intramuscular arterioles. In our cases of rheumatoid arthritis *early* stages of muscular degeneration were seen only when there was a severe endomysial inflammation; without inflammation no beginning muscle fiber degeneration was found. Thus, muscular degeneration in rheumatoid arthritis does not give rise to inflammation; rather, muscular degeneration is a consequence of the inflammatory nodular lesions because of the close spatial relationship of damaged muscle fibers to inflammatory foci. The irregular distribution of degenerated muscle fibers in close relationship to inflammatory lesions, the appearance of débris of muscle fibers in the nodules, and the absorptive phenomena of muscular tissue in intimate connection with the nodular inflammation are proof that muscular degeneration and atrophy are a consequence of the nodular inflammatory polymyositic process.

It may be asked why a similar secondary degeneration of the nerve fibers in peripheral nerve bundles was not found accompanying the perineuritic nodular inflammation in our previously reported cases. Endomysium, perimysium, and muscle fibers are all of mesodermal origin. Consequently an inflammatory process encroaching on the muscle fibers by way of the perimysium and endomysium does not encounter such a complete barrier as it does in the perineurium at the border between mesodermal and ectodermal tissues of the peripheral nerves. As a result of this barrier endoneurial inflammatory lesions were not seen or were extremely rare while endomysial inflammation was com-

mon. With perineuritic inflammation no degenerated nerve fibers or endoneurial inflammation could be seen, while endomysial and perimysial nodular inflammation with damage to the muscle fibers was prominent in the same section. The occurrence of both types of inflammation in the same section indicates that the inflammatory processes are identical and that the resistance of nerve fibers is due to histological and physiological dynamics by which the nerve fiber is much better protected than the muscle fiber.

In summary, the inflammatory lesions in the synovia, joints, and capsular and pericapsular tissues; the subcutaneous nodules; the nodular lesions in peripheral nerve trunks; and the nodular polymyositic inflammation with arteritis and perineuritis in skeletal muscles are concomitant lesions due to the same unknown cause but entirely independent of one another. They are coordinate, not subordinate. However, muscular degeneration is a consequence of the inflammatory lesion in the perimysium and endomysium.

Clinical Significance of Nodular Polymyositis in Rheumatoid Arthritis

Clinically, rheumatoid arthritis has to be treated as a chronic inflammatory process, for the similar character of the muscular lesions and the lesions in other structures (synovia, subcutaneous tissue, and perineurium) is strong evidence of a generalized inflammatory process, possibly of infectious origin. Since the infectious agent is unknown, only general principles of treatment can be applied. That gold (myochrysine) treatment is not entirely satisfactory is evident from the fact that muscle specimens taken from cases 3, 5, and 6, thus treated, revealed inflammatory nodules identical with those seen in our cases in which no gold treatment had been given recently or in which inadequate amounts of myochrysine had been administered several years before (case 4).

Polymyositic nodules are specific for rheumatoid arthritis. In the future the clinical diagnosis in early or doubtful cases of rheumatoid arthritis may be supported by biopsy of affected muscles. Further, it is possible that the value of therapeutic procedures may be controlled by such means.

The findings in rheumatoid arthritis are more closely related to those of rheumatic fever than to those of any other disease. Whether there is an etiological relationship between these two diseases cannot be established by morphological investigation. However, when both have been recognized in the same patient, rheumatic fever has always preceded rheumatoid arthritis.

Rheumatoid arthritis, even in its late stage, is not a disease that has terminated in scar formation but is a smoldering, active, distinctive entity as shown by the presence of inflammatory foci in the muscles and in the perineurium. The seemingly "burnt-out" clinical picture in old cases of rheumatoid arthritis without pains but with extreme stiffness in joints and with bony deformities is deceptive, since active inflammatory processes are found in the muscles and the peripheral nerves.

Stiffness and bony contractures are responsible for some limitation of muscular activity. However, we wish to emphasize our conclusion that the anatomopathological lesions herein described, rather than disuse, are responsible for the clinical picture of muscular atrophy in rheumatoid arthritis.

The multiplicity of the inflammatory lesions in the synovia and other articular and periarticular structures, in the peripheral nerves, and in subcutaneous tissues indicates a wide spread of the causal agent in cases of rheumatoid arthritis. With the recognition of nodular polymyositis a new link in this nosological chain has been found. Simultaneously, the primary inflammatory character of this disease is made more evident.

Summary

1. The pathological findings reported in this paper comprise "nodular polymyositis." Together with previously reported perineuritis, it constitutes an essential lesion in rheumatoid arthritis, to be called nodular neuromyositis.

2. The muscular lesions are specific for rheumatoid arthritis, since they were seen in every one of the 9 cases of rheumatoid arthritis and since they were absent in control material from 196 routine autopsies of cases of other diseases and 7 selected cases in which pathological lesions in muscles could be expected.

3. The distribution of the lesions in small amounts of various muscles (gastrocnemius, other unidentified muscles of the legs (case 7), triceps, deltoid, pectoralis major, and rectus abdominis) taken at random, and the high incidence of the lesions in a minimal percentage of the entire skeletal muscular system indicate the wide spread of nodular polymyositis throughout the skeletal muscular system.

4. The muscular lesions fit into the general pattern of other lesions known in rheumatoid arthritis, such as those seen in the synovia and other tissues of the joints, in subcutaneous nodules, and in the perineurium of peripheral nerve trunks. They are all alike in their inflammatory and granulomatous nature. The muscular lesions differ from those seen in other diseases (myatonia congenita, muscle dystrophy,

myasthenia gravis, dermatomyositis, "fibrositis," trichinosis). Besides the endomysial location of the lesions, perivascular and perineuritic nodular arrangements are seen in the perimysium.

5. The muscular lesions are not related spatially to lesions of the synovia, the periarticular tissues, the subcutaneous nodules, or of the peripheral nerves. They are concomitant but independent tissue reactions to the same unknown agent. Our material is not complete enough to establish a chronological order for these various manifestations. However, it seems evident to us that the muscular involvement begins early, perhaps at the same time that lesions of the joints become manifest (see case 5).

6. There is a definite pathogenetic relationship between nodular polymyositis and degeneration and atrophy of the muscle fibers. While inflammation can occur without degeneration of muscle fibers, early muscle fiber degeneration is always combined with inflammatory lesions in the endomysium of the same fibers. Hence an irregular distribution of degenerated muscle fibers is the consequence of irregular spread of nodular inflammation. The absorption phenomenon of degenerating muscle in intimate relation with the nodular inflammatory foci is further evidence of the primary nature of the inflammation and the secondary nature of muscle fiber degeneration.

7. Various stages of muscle fiber degeneration are recognizable from the early change in staining quality; through invasion of the contractile substance by peculiar nuclei and the appearance of vacuolization, irregular outer contour, and irregular cross striation; to extreme shrinkage of the muscle fibers. This sequence seems to be related to the duration of the disease or to the time of the involvement of the particular muscle examined.

8. From a clinical standpoint our pathological findings are important as evidence of the wide distribution of the inflammatory and probably infectious process throughout the body in rheumatoid arthritis. The finding of nodular polymyositis in cases treated with gold compounds leads to the conclusion that our present treatment is not sufficiently effective. The presence of polymyositic nodules in old clinically "burnt-out" cases is evidence of a permanent and active disease process. In the future, biopsy of muscle may be an aid in diagnosis, particularly in early cases, and in the control of chemotherapeutic action.

We wish to express our gratitude to Drs. Charley J. Smyth and S. E. Gould of Eloise Hospital, Eloise, Michigan, and to Dr. Plinn Morse of the Department of Pathology at Harper Hospital, Detroit, Michigan, for granting the use of material.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

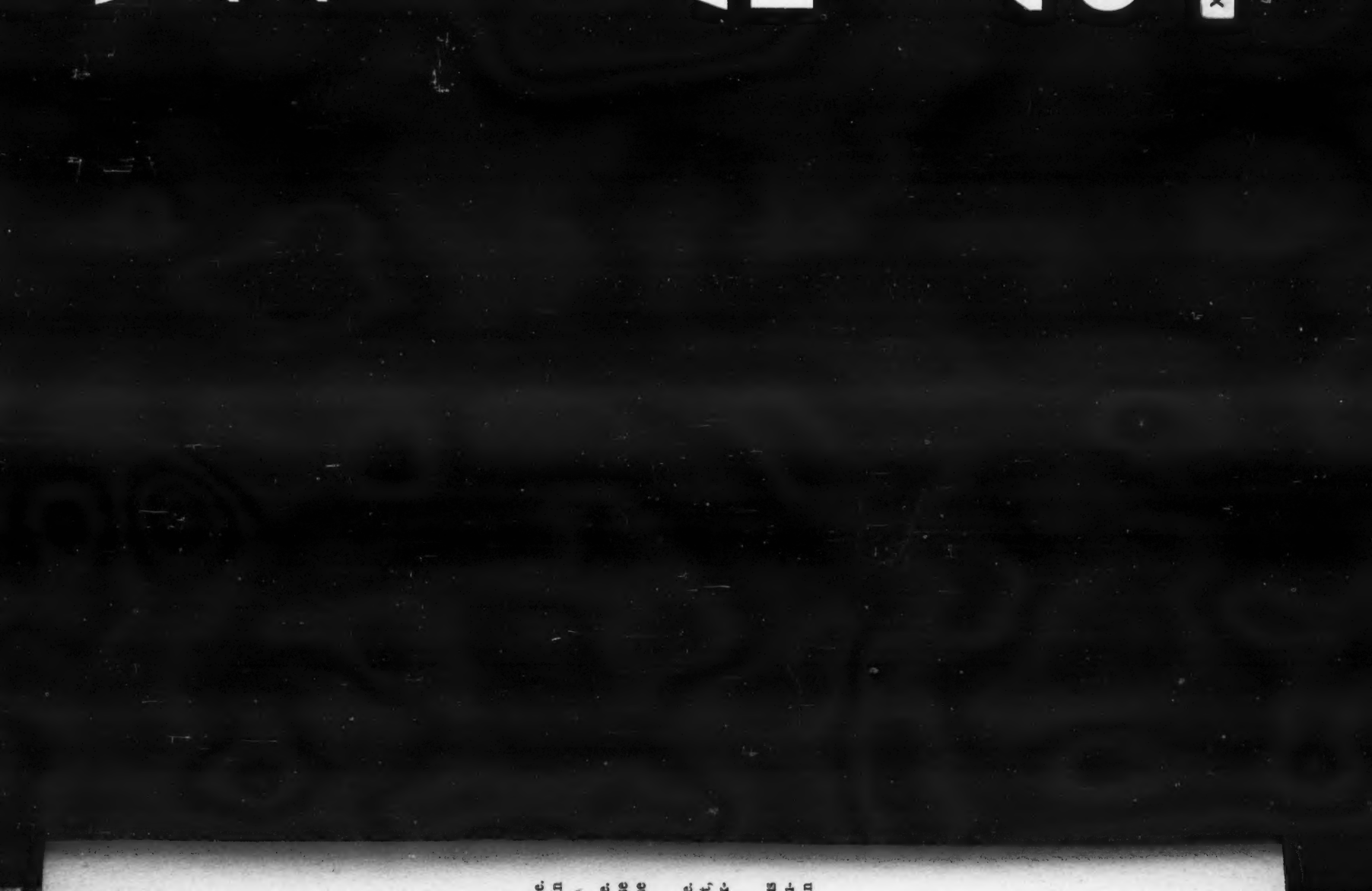
PLATE 22

FIG. 1. Case 1. Specimen removed for biopsy from the gastrocnemius muscle. Spindle-shaped inflammatory nodule in endomysium. Hematoxylin and eosin stain. × 100.

FIG. 2. Case 1. Specimen removed for biopsy from the gastrocnemius muscle. Smaller, triangular shaped, inflammatory nodule. Small amount of adipose tissue between two muscle fibers at the right lower angle of the nodule. At the upper end a pale muscle fiber is seen. Hematoxylin and eosin stain. × 300.

FIG. 3. Case 1. Specimen removed for biopsy from the gastrocnemius muscle. General increase in sarcolemmal nuclei. Large muscle fibers, partially crosscut, are seen in the center of the field. Between them there are a number of extremely shrunken fibers. Hematoxylin and eosin stain. × 100.

FIGS. 4 and 5. Case 1. Specimens removed for biopsy from the gastrocnemius muscle. Inflammatory nodules in a perimysial location adjacent to intramuscular nerves and to the adventitia of blood vessels. Hematoxylin and eosin stain. × 100.



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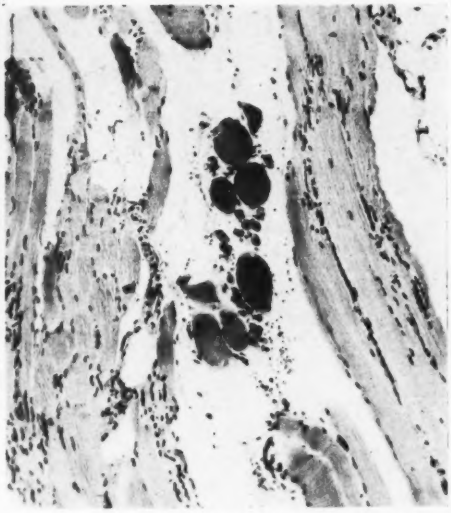

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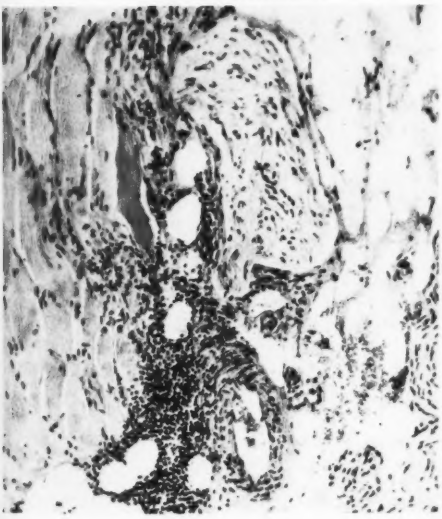

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PLATE 23

FIG. 6. Case 2. Specimen removed for biopsy from the gastrocnemius muscle. Longitudinally cut, markedly atrophic muscle fibers are shown to the right and below three swollen, discolored, and slightly vacuolated fibers. Hematoxylin and eosin stain. × 150.

FIG. 7. Case 2. Specimen removed for biopsy from the gastrocnemius muscle. Inflammatory nodule in perimysial location between two small intramuscular nerves. Hematoxylin and eosin stain. × 150.

FIG. 8. Case 4. Specimen removed for biopsy from the triceps muscle. Well circumscribed, endomysial, nodular inflammation with extensions of infiltrating cells from the body of the nodule. Hematoxylin and eosin stain. × 150.

FIG. 9. Case 4. Specimen for biopsy from the triceps muscle. A large inflammatory nodule with accumulations of epithelioid cells near its periphery. Cresyl violet stain. × 150.

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PLATE 24

FIG. 10. Case 4. A smaller inflammatory nodule from a specimen from the triceps muscle. There are many scattered mast cells. Cresyl violet stain. × 150.

FIG. 11. Case 4. Specimen removed for biopsy from the triceps muscle. Beginning degeneration of a muscle fiber, marked by lighter staining, and central position of muscle nuclei of larger size and having two nucleoli. The perinuclear sarcoplasm is granular and shows beginning vacuolization. The endomysium near this muscle fiber shows infiltration with lymphocytes and plasma cells. Van Gieson's stain. × 675.

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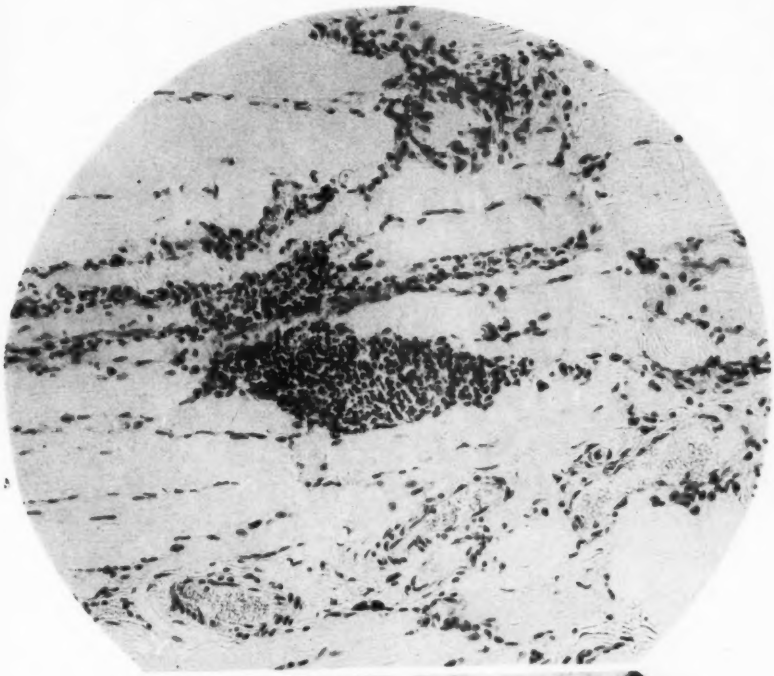

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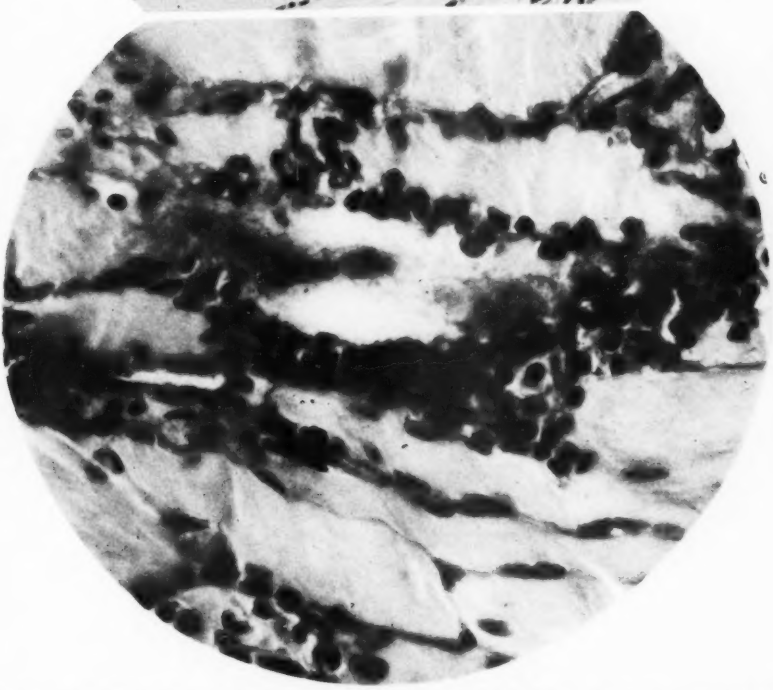

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PLATE 25

FIG. 12. Case 4. Specimen removed for biopsy from the triceps muscle. Two muscle fibers, showing degeneration, have a nodular inflammatory reaction around them, and there is a longitudinal arrangement of small vacuoles in the smaller of the two fibers. There are, also, loss of cross striation, and a pale-staining quality. Van Gieson's stain. × 675.

FIG. 13. Case 4. Specimen removed for biopsy from the triceps muscle. Abnormal nuclei at the periphery of a degenerating muscle fiber which is surrounded by lymphocytes and plasma cells. Van Gieson's stain. × 675.

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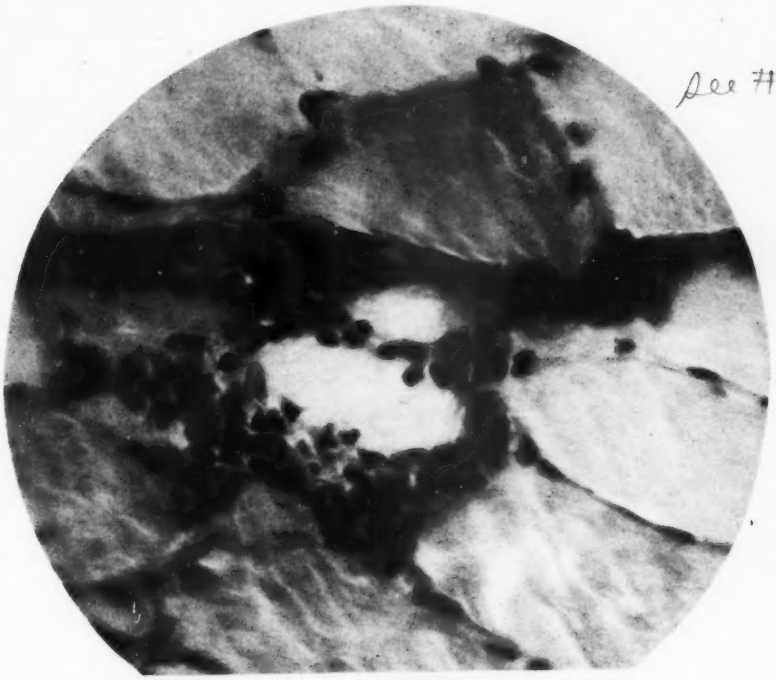

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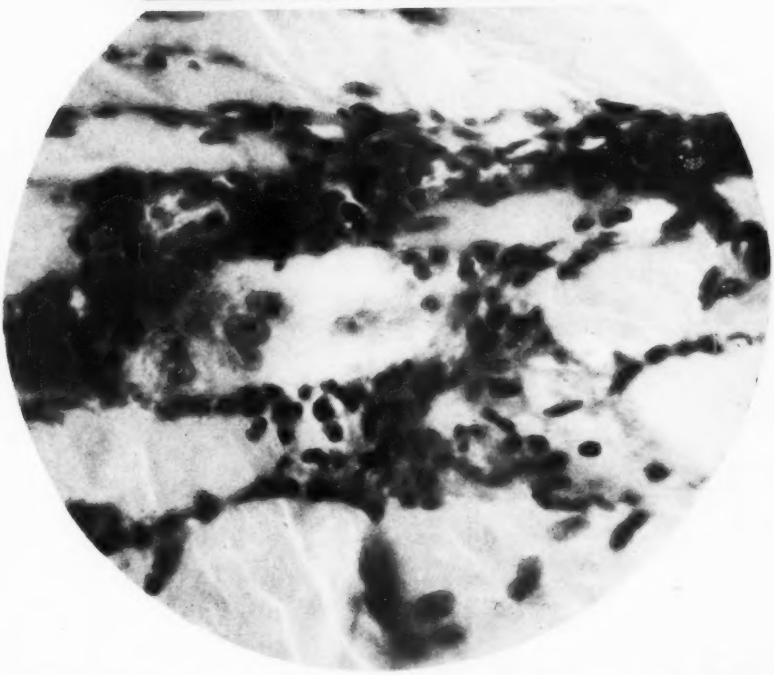

PLATE 26

FIG. 14. Case 4. Specimen removed for biopsy from the triceps muscle. Two degenerating muscle fibers surrounded by an area of endomysial inflammation. There is a large, dark-staining nucleus in one of the fibers. Van Gieson's stain. × 600.

FIG. 15. Case 4. Specimen for biopsy from the triceps muscle. Crossing the field there is a degenerating muscle fiber with coarse cross striations. Below this, a second fiber shows beginning longitudinal fibrillation. This preparation was stained with cresyl violet, which gives injured muscle fibers a deeper blue color than that taken by normal fibers. × 675.

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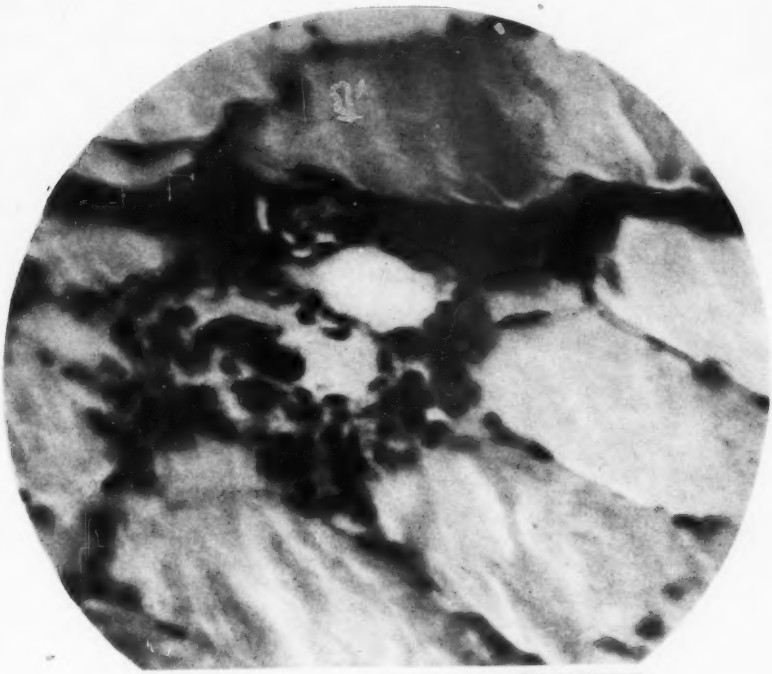

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PLATE 27

FIG. 16. Case 4. Coarse, wavy cross striation of a muscle fiber. Infiltration of lymphocytes and plasma cells. Cresyl violet stain. × 675.

FIG. 17. Case 5. Specimen removed for biopsy from the gastrocnemius muscle. Small nodular inflammatory focus in an endomysial location. This shows the extent of infiltration in a transverse plane. Hematoxylin and eosin stain. × 100.

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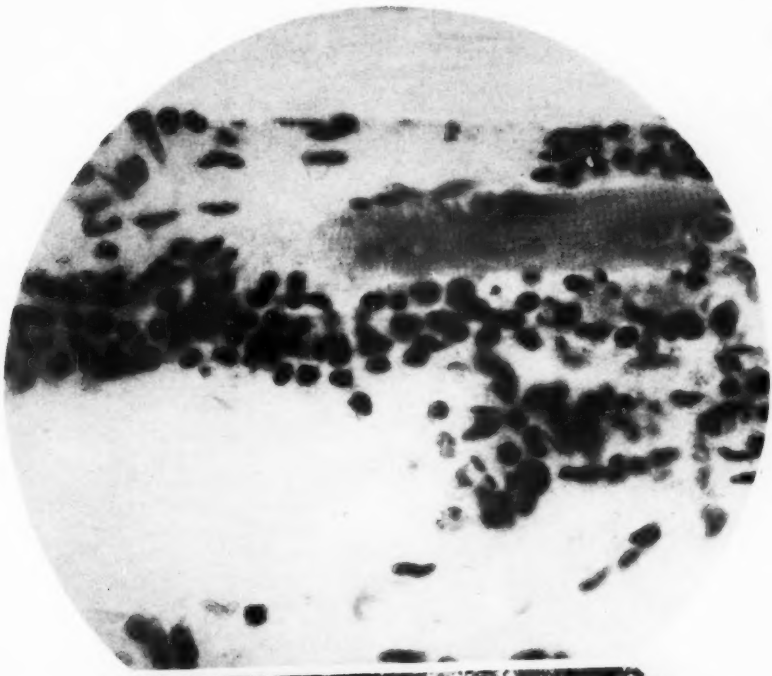

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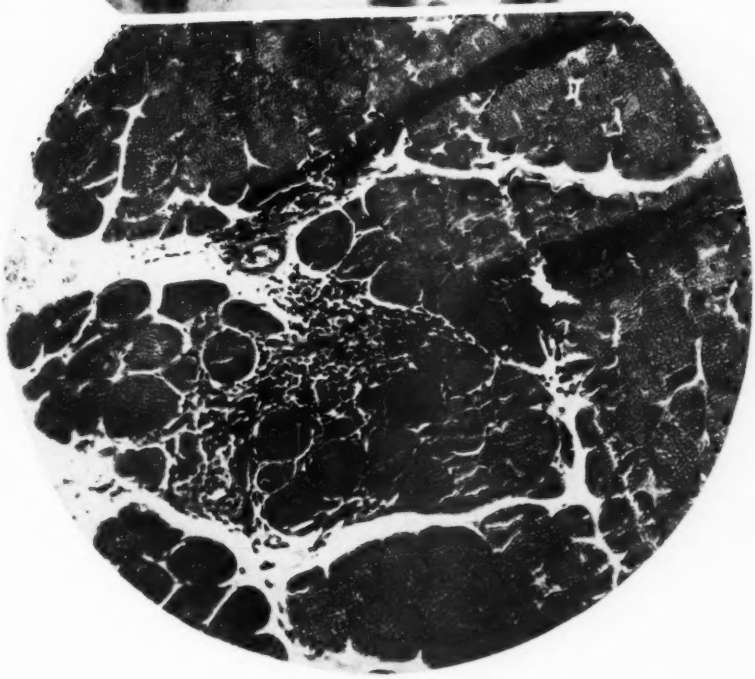
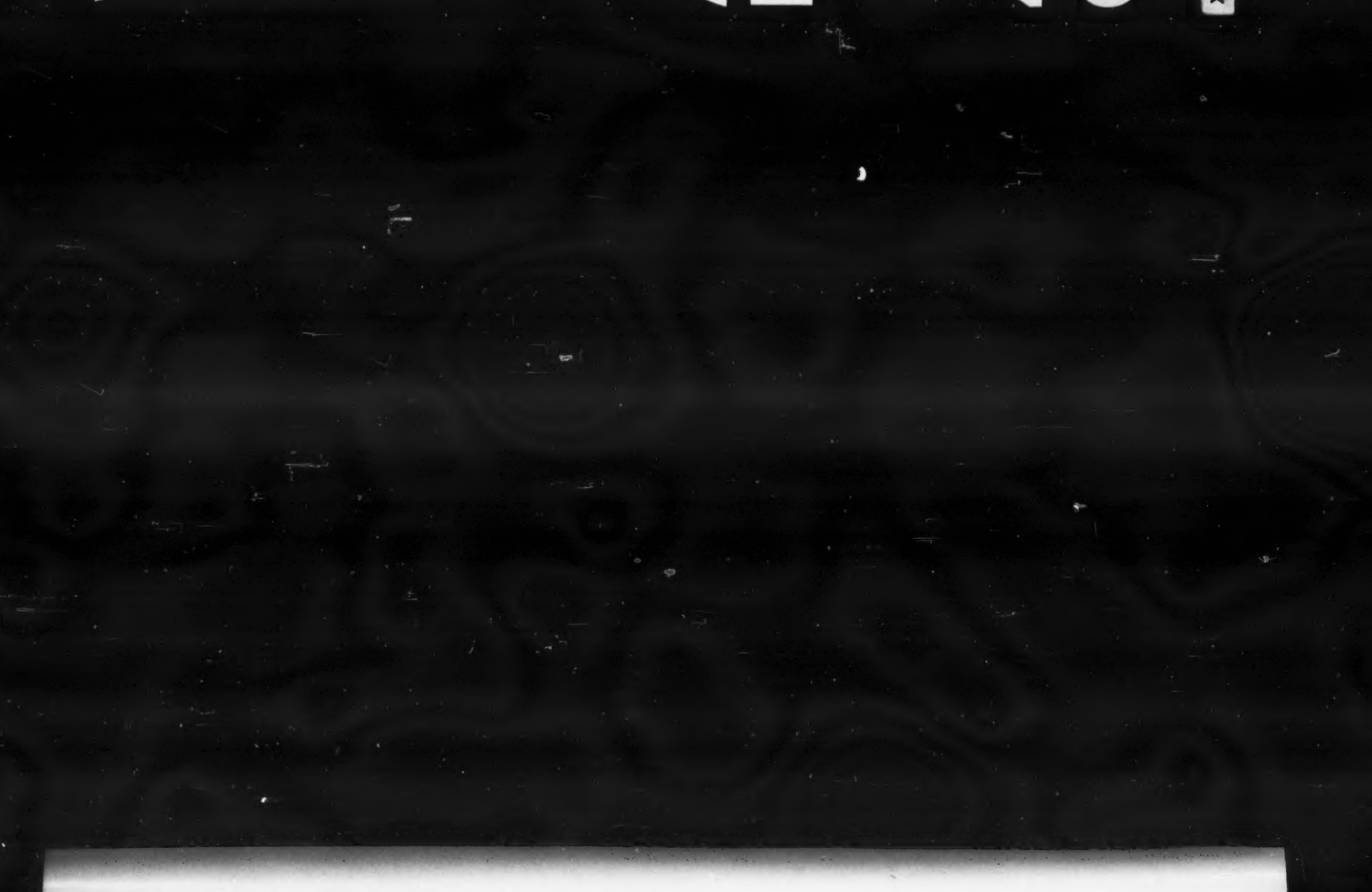


PLATE 28

FIG. 18. Case 5. Specimen removed for biopsy from the gastrocnemius muscle. A degenerating muscle fiber, with a more centrally placed row of muscular nuclei and vacuolization at the periphery and around the large nuclei, crosses the lower center of the field. Hematoxylin and eosin stain. × 675.

FIG. 19. Case 7. Specimen obtained following amputation of both legs. Muscle tissue near the tibial nerve with a massive inflammatory nodule and severe degeneration and atrophy of muscle fibers. The inflammatory cells are lymphocytes and plasma cells. Hematoxylin and eosin stain. × 100.

FIG. 20. High-power photomicrograph of a portion of the field of Figure 19, showing remnants of degenerating muscle fibers between lymphocytes and plasma cells. Hematoxylin and eosin stain. × 450.



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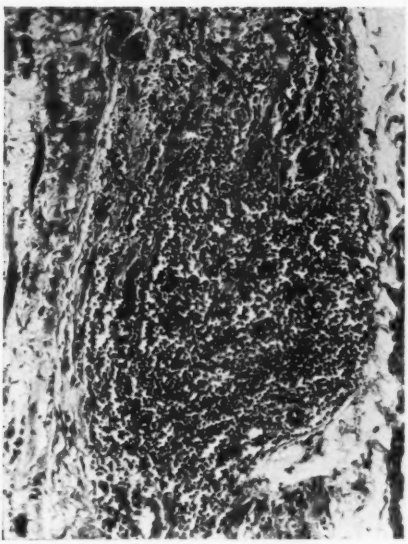

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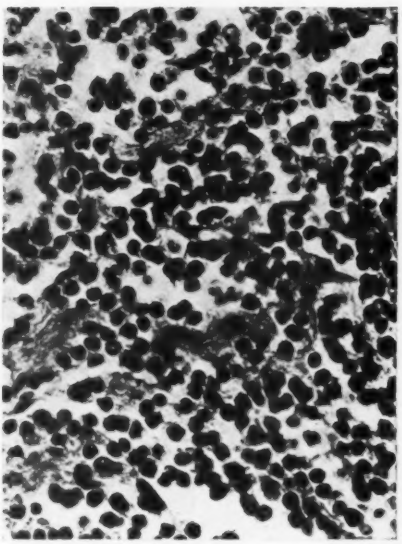

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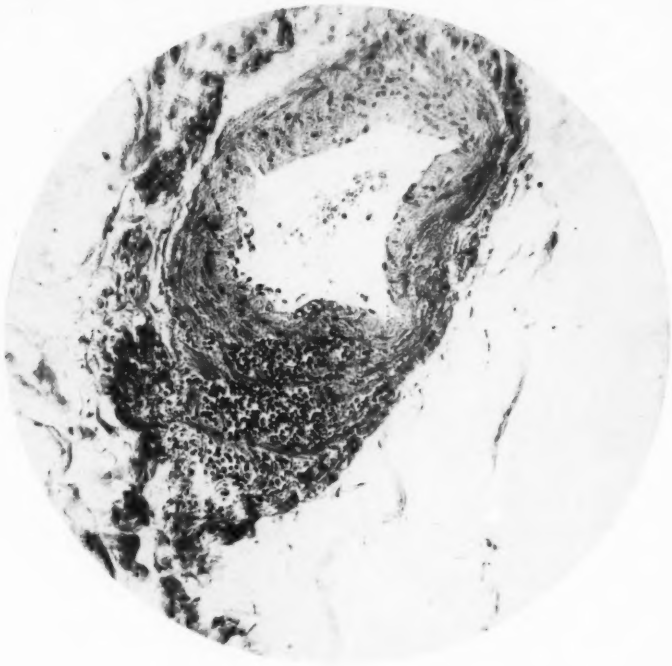
PLATE 29

FIG. 21. Case 7. Specimen obtained following amputation of both legs. Nodular lymphocytic inflammation in the wall of an artery (media and adventitia), located in perimysium. Hematoxylin and eosin stain. × 150.

FIG. 22. Case 7. A higher power photomicrograph of a portion of the field shown in Figure 21. Hematoxylin and eosin stain. × 450.

FIG. 23. Case 7. Muscle tissue found in the vicinity of the left superficial peroneal nerve. Nearly complete lymphocytic infiltration of the perineurium of a small intramuscular nerve. Hematoxylin and eosin stain. × 150.



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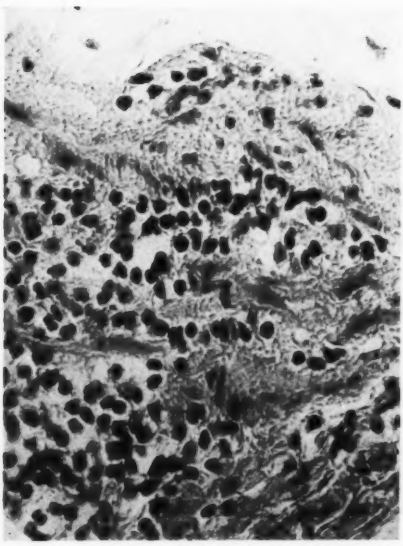

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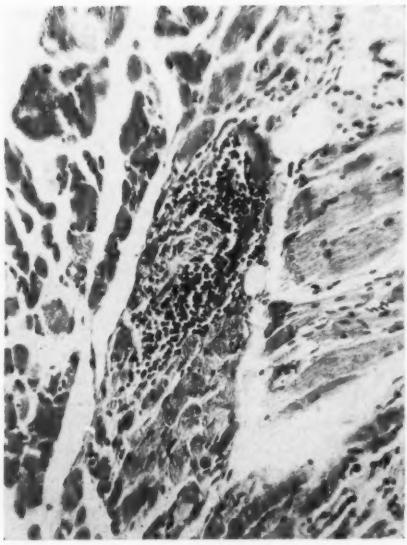

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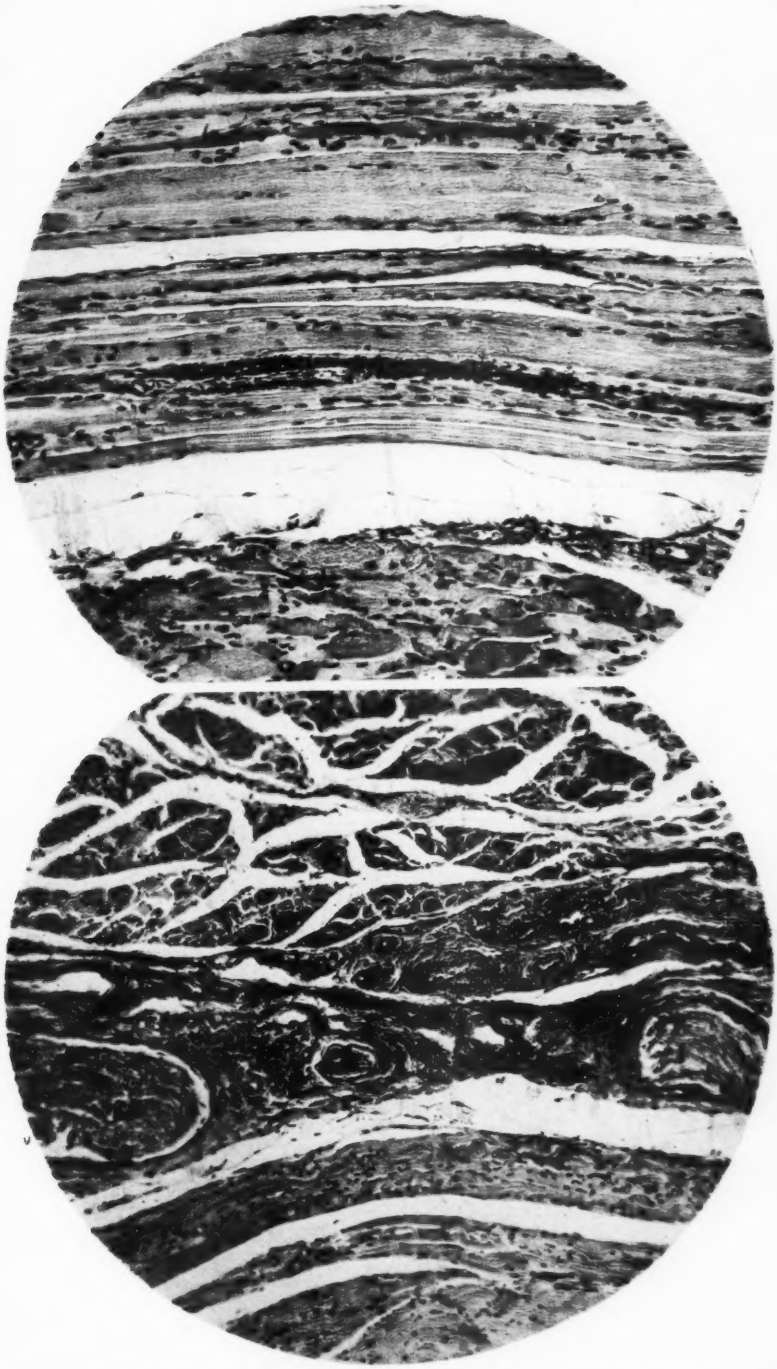
PLATE 30

FIG. 24. Case 8. Necropsy specimen from pectoralis major muscle. Small, elongated, spindle-shaped, circumscribed, lymphocytic inflammatory focus in the endomysium. Hematoxylin and eosin stain. × 150.

FIG. 25. Case 8. Necropsy specimen from the pectoralis major muscle. Two perimysial inflammatory nodules in periadventitial and perineurial locations. Hematoxylin and eosin stain. × 125.



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HYDROGEN SULFIDE POISONING
Report of Two Cases, One with Fatal Outcome, from Associated Mechanical Asphyxia *

A. W. Freireich, M.D.†

(From the Office of the Chief Medical Examiner (Dr. Theodore J. Curphey), Nassau County, N.Y.)

Poisoning by hydrogen sulfide is a rather unusual occurrence. In certain industries, such as mining gypsum and sulfur, production and refining of high sulfur petroleum, and the manufacture of chemicals, dyes and pigments, hydrogen sulfide is encountered. A group of cases of local and systemic hydrogen sulfide intoxication in workers engaged in excavating in the oolithic limestone of southern Florida has recently been reported.[1]

In large urban centers hydrogen sulfide poisoning is extremely uncommon. Gettler [2] stated that only one such case had been observed by the office of the Chief Medical Examiner of New York City in 25 years.

The following two cases are reported because of the unusual circumstances and in order to acquaint the medical profession and the public with the hazards existing in suburban areas where sewage disposal is by means of cesspools.

Report of Cases

The cesspool in the rear yard of the house of Mr. A. G., a white male, 42 years old, became filled and was not functioning properly. He engaged a firm of cesspool cleaners to remedy the situation. This was done by pumping approximately half of the fluid content from the pit, which was 8 ft in diameter and 10 ft. deep, and then adding the contents of a large demijohn to the remainder. (The liquid that was added was later found to be commercial concentrated sulfuric acid.) The cesspool was capped and the workers left the premises.

The next morning, Mr. A. G. investigated and found fluid remaining in the cesspool to a level of 1½ ft. He decided, with the help of his son, A. G., Jr., 17 years old, to empty the remainder with a bucket tied to a rope. When the fluid level became too low for the bucket to be effective, Mr. A. G. lowered himself into the cesspool, filled the bucket, and had his son hoist it up. After a short while in the pit he noticed a smarting of the eyes and returned to the ground level. He entered the pit three times and each time felt a burning sensation about his eyes. After the fourth descent he felt weak and called to his son to "get the ladder down, quick!" He lost consciousness and, on reviving, saw his son lying on the floor of the cesspool. He attempted to move the boy, but again became unconscious. On reviving a second time he called for help and both were removed by neighbors. The father recovered rapidly but the boy remained unconscious and died despite continued artificial respiration.

An autopsy was performed 20 hours after death. On external examination the head and face showed a peculiar greenish blue cyanosis.

* Received for publication, February 15, 1945.

† Deputy Medical Examiner and Toxicologist, Nassau County, N.Y.; Toxicologist, Meadowbrook Hospital, Hempstead, N.Y.

There were also innumerable small, darker green, circular areas scattered in the greenish blue field (Fig. 1). These spots were located mainly over the left side of the forehead, left cheek and chin. Over the bridge of the nose, across the lips, and on the tip of the chin there were brownish areas having the appearance of corrosive acid burns. The exposed portions of the wrists and hands showed a greenish blue cyanosis similar to that on the face, with darker green, circular areas. The skin of the thighs and legs showed the greenish blue color that is usually seen in advanced decomposition (Fig. 2). The suggillations in some areas were purplish red, but in others were greenish blue.

On making the main incision, pieces of filter paper moistened with freshly prepared lead acetate solution were placed in contact with the pectoral muscles. A faint yellowish brown stain appeared.

When the lungs were removed, a strong odor of hydrogen sulfide was perceived and small bubbles of the gas were seen to escape from the cut ends of the bronchi. Lead acetate paper held near the cut end of the bronchus turned a brown to black color (Fig. 4). On dissecting the bronchi there was seen a large amount of brownish, sandy material mixed with sewage extending to the bronchi of the third and fourth order. Considerable pulmonary edema and congestion were present as well as several large subpleural hemorrhages.

The mouth was filled with brownish, gritty sewage material mixed with sand. The tongue appeared as if burned with acid and a similar picture was seen in the epiglottis, larynx, pharynx, and hypopharynx (Fig. 3). The esophagus throughout its entire length showed a whitish slough on the mucous surface as from acid digestion.

The remainder of the autopsy revealed nothing noteworthy.

Microscopic examination of sections of lung tissue revealed rather marked congestion and exudation of serous fluid into the alveolar spaces. The spleen and kidney showed congestion and the appearance of the other tissues was normal.

Chemical examination of the material removed from the cesspool proved the presence of a large quantity of hydrogen sulfide and free sulfuric acid.

Spectroscopic examination of the blood failed to reveal the characteristic bands of either sulfhemoglobin or methemoglobin.

Discussion

That sewage which is allowed to putrefy without the access of air is subject to the formation of hydrogen sulfide by the action of bacteria is a well known fact.[3] The sulfides are kept in solution by alkaline salts. The addition of a large quantity of sulfuric acid liberates free hydrogen sulfide.

Hydrogen sulfide is a colorless gas with a specific gravity of 1.192 and an odor as of rotten eggs, familiar to everyone who has worked in a qualitative chemical laboratory. However, a less well known fact is that as the concentration of the gas is increased the odor becomes less apparent, due to paralysis of the olfactory nerves.[4]

In the accident which I have described, there was apparently no awareness of any unusual odors. On being questioned after recovery, the father stated that he did not notice the odor, when it was described to him. Furthermore, when I visited the scene shortly after the boy was pronounced dead, I did not note the odor and it was only after I had walked approximately 50 ft. from the cesspool that the nature of the gas became apparent.

Both the father and son were overcome by the gas. The father became conscious long enough to be saved. The son, however, possibly through the increased respiration engendered by the activity in trying to help his father, became unconscious rapidly and fell face down into the pool of sewage, aspirating the material into his air passages, and finally died as a result of hydrogen sulfide poisoning and mechanical obstructive asphyxia.

The concentrations of the gas in the respired air corresponding to the various manifestations of the poisoning have been determined.[3-5] Table I is that reported by Haggard [4] and is in fairly close agreement with the others.

TABLE I

The Toxic Concentrations of Hydrogen Sulfide *

Concentration of hydrogen sulfide in air			Symptomatology
Mg. per liter	Parts per million	Per cent	
0.14 to 0.21	100 to 150	0.01 to 0.015	Symptoms of local irritation after many hours of exposure.
0.28 to 0.42	200 to 300	0.02 to 0.03	Causes local irritation if inhaled for one hour and slight general symptoms if inhaled longer.
0.70 to 0.97	500 to 700	0.05 to 0.07	Causes local irritation and slight systemic symptoms within one hour. May cause death after exposure of several hours.
1.0	900	0.09	Causes systemic symptoms in less than thirty minutes. May cause death in less than one hour.
1.7	1,500	0.15	Causes death after fifteen to thirty minutes of exposure.
2.0 and over	1,800 and over	0.18 and over	Causes almost immediate death through paralysis of breathing.

* Reprinted, by permission, from Haggard, H. W. The toxicology of hydrogen sulphide. *J. Indust. Hyg. & Toxicol.*, 1925, 7, 113–121.

The determination of the cause of death by finding hydrogen sulfide in the blood is impossible as the "oxidation is of such rapidity that the gas disappears during the time spent in making the necessary manipulation." [4]

Spectroscopic examination of the blood is unreliable.[6] Sulfhemoglobin has never been found in the blood of persons who have died from hydrogen sulfide poisoning when the examination was made before decomposition had set in.[4] When brought into contact with blood in which the hemoglobin contains oxygen, the hydrogen sulfide is oxidized and no sulfhemoglobin is formed. Only when fresh hydrogen sulfide is brought to act on the blood after complete reduction has occurred, as happens during putrefaction, is a sulfhemoglobin compound formed.

In the fatal case reported here, determination of the actual causative agent was aided by the presence of material containing hydrogen sulfide in the bronchi and examination of the scene shortly after death was reported. The reaction obtained by placing lead acetate paper in contact with the sectioned muscles,[6] while providing valuable corroboration, cannot be used as conclusive proof.

Summary and Conclusions

Hydrogen sulfide is a very toxic gas. At concentrations at which the odor is apparent, the toxicity is not very great, but when higher concentrations are reached rapid poisoning may occur. The danger becomes greater because of the early paralysis of the olfactory nerves and consequent unawareness of his peril on the part of the subject.

Two cases of hydrogen sulfide poisoning are reported, one with fatal outcome, as a result of attempting to clean out a cesspool to which concentrated sulfuric acid had been added. Health protection agencies and the public should be warned of the dangers inherent in such procedures.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 31

FIG. 1. In addition to the greenish blue and dark green areas distributed particularly over the left side of the forehead, left cheek and chin, there are brownish areas due to contact with sulfuric acid.

FIG. 2. Fatal hydrogen sulfide poisoning, with associated obstructive asphyxia. The skin of the face, wrists, hands, thighs, and legs showed a peculiar greenish blue color with small, darker green, scattered, circular areas.

1

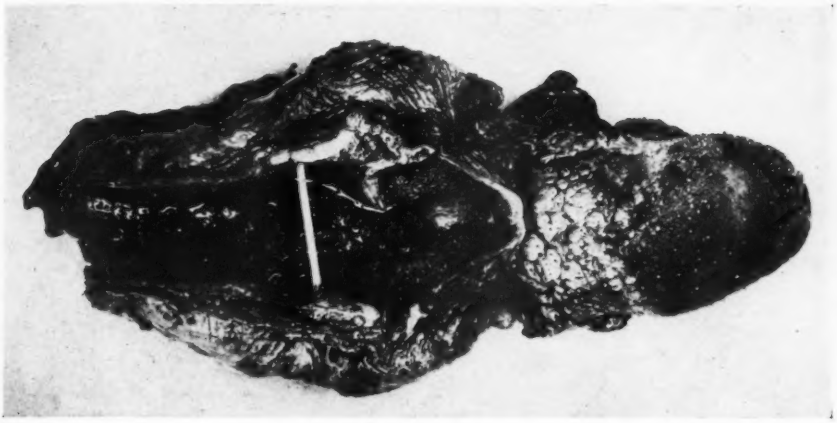
2

Freireich Hydrogen Sulfide Poisoning

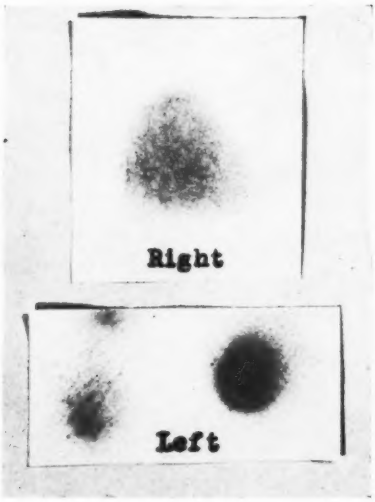
PLATE 32

FIG. 3. Sulfuric acid burns of the tongue, pharynx, epiglottis, and larynx.

FIG. 4. Stains obtained by holding lead acetate paper near the cut ends of the right and left bronchi.



3



4

Freireich Hydrogen Sulfide Poisoning

HYPERPLASIA OF THE ADRENAL CORTEX ASSOCIATED WITH BILATERAL TESTICULAR TUMORS *

HILLIARD COHEN, MAJOR, M.C., A.U.S.†

Hyperplasia and neoplasia of adrenocortical tissue are commonly associated with profound alterations in somatic and genital development.[1-6] In the prepubertal male these alterations usually are manifested by excessive masculinization, including precocious development of primary and secondary sexual characteristics and increased body growth. As far as can be ascertained, the literature does not contain a single report of proved adrenocortical hyperplasia or neoplasia with macrogenitosomia in the male, in which the patient lived to adult life. Only one instance of a combination of hyperplasia of the adrenal cortex, hyperplastic adrenocortical tissue in the testes, and macrogenitosomia has been previously reported.[1] This patient was a child who died at the age of 3½ years.

I wish to report a case, with autopsy findings, of bilateral hyperplasia of the adrenal cortex associated with bilateral, solitary, testicular masses resembling adrenocortical tissue, occurring in an adult male who in childhood exhibited pronounced precocious genital and somatic development.

REPORT OF CASE

Clinical History. The patient was an acutely ill white male, 20 years old. He was admitted to the Station Hospital, Fort Belvoir, Va., with a 24-hour history of fever, chilliness, headache, right chest pain, nausea, and vomiting. On examination, the temperature was 104° F., respirations were 28, pulse was rapid and barely perceptible, and blood pressure was 60/40 mm. of Hg. Shortly after admission the blood pressure was unobtainable, and the patient died 12 hours later in profound peripheral circulatory failure. The vascular collapse dominated the clinical picture. The clinical diagnosis was lobar pneumonia, lower lobe, right lung, pneumococcus, type 1. Cultures of the blood and spinal fluid were sterile.

A developmental abnormality was suggested by the striking findings in the adrenal glands and testes at post-mortem examination. Through the cooperation of the mother of the patient, valuable records and a snapshot of the patient at the age of 2½ years were obtained from the pediatrician (Dr. Pounders) who had attended the patient from the age of 20 to 35 months. In addition, Mt. Sinai Hospital of Cleveland, Ohio, forwarded a clinical abstract of the examinations of the patient when he was 6 years of age.

When first seen by the pediatrician, the boy was 20 months of age, of normal height—32¾ inches (83 cm.)—and slightly underweight (Table I). He appeared normal in all other respects except for evi-

* Received for publication, February 19, 1945.

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dence of slight rickets. No abnormalities in somatic development or in development of the sexual organs were apparent at that time. At a subsequent visit 9 months later (at 29 months of age, Fig. 1), the penis was greatly enlarged, and the pubic hair was about ½ inch (1.3 cm.) in length. A history of frequent erections was obtained. The height was 34½ inches (88 cm.), which was still within normal limits for his age. When last seen by the pediatrician the boy was 35 months of age; his height was 39¾ inches (101 cm.), an increase of 5¼ inches (13 cm.) in 6 months. The penis was still distinctly overdeveloped, and frequent erections persisted. The testes were not proportionately enlarged. Radiographic examination of the skull revealed no abnormalities.

TABLE I

Height and Weight of Patient at Different Ages, as Compared with Approximate Average Normals for the Same Ages

Age	Height		Weight	
	Patient	Average normal*	Patient	Average normal*
	inches	inches	pounds	pounds
20 mos.	32¾	32		
29 mos.	34½	35½		
35 mos.	39¾	37		
6 yrs.	56¾	45	78	44
20 yrs.	61	68	140	135

*Normal values derived from: Holt, L. E., and McIntosh, R. Diseases of Infancy and Childhood. D. Appleton-Century Co., Inc., New York, 1940, ed. 11, p. 22.

At the age of 6, the boy was taken to the Out-Patient Department of the Mt. Sinai Hospital of Cleveland. At that time his height was 56¾ inches (144 cm.), an increase of approximately 1½ feet (43 cm.) in 3 years, and his weight was 78 pounds (35 kg.). The external genitalia were well developed, and there was a moderate growth of pubic hair. The physical development was that of a boy of 10 to 12 years. The head was "somewhat suggestive of hydrocephalus." Thorough medical, neurologic, and ophthalmologic examinations revealed no further significant findings. There were no pathologic changes in the cranial cavity on radiographic examination. The basal metabolic rate was within normal limits (plus 11 per cent); the glucose tolerance curve was normal; and routine examinations of blood and urine were negative.

Further pertinent information from medical sources was not obtained. According to the mother, the period of rapid growth lasted until the boy was 10 years of age, and very little growth, if any, occurred after the age of 12. At that time he was approximately 5 feet (152 cm.) tall. The mother further stated that the boy's mental de-

velopment kept abreast of his rapid physical development, and "he seemed to have a clearer understanding and a more balanced sense of values than the average boy of his age group." There was no record of a craving for salt or salty foods.

Statements prepared by soldiers closely associated with the patient during his period of army service indicated: (1) that his intelligence was well above average, (2) that he mixed well with the soldiers and was uniformly popular among them, (3) that he apparently did not suffer an inferiority complex because of his short stature, (4) that he overcompensated for his small size by physical vigor, which led on occasions to evidence of extreme tiredness, (5) that he was religious, and closely attached to his mother, (6) that he did not engage in conversations regarding sex to the same extent as most of the other men, and (7) that he had never had sexual intercourse.

POST-MORTEM EXAMINATION *

The body was well developed and well nourished, with a normal but small male physique and with normal male distribution of hair. There was no gross disproportion between length of trunk and of the extremities. The body length was 61 inches (155 cm.). The body weight was estimated to be 140 pounds (64 kg.). The scalp was covered with a scant growth of thin brown hair. No abnormal pigmentation of the skin or mucous membrane was noted. The penis was of average size. Both testes were in the scrotum, and by palpation each seemed smaller than usual. There were no abdominal striae, and excessive subcutaneous fat was not present. The pertinent findings exclusive of the suprarenal glands and testes were:

(1) Type 1 pneumococcus lobar pneumonia, middle lobe, right lung;

(2) Persistent thymus of moderate size (35 gm.), moderate lymphoid hyperplasia of the appendix, and slight lymphoid hyperplasia of the spleen. Lymphoid tissue elsewhere was not hyperplastic;

(3) Cholelithiasis—solitary, soft, brown-black calculus containing a large amount of bile pigments, a trace of cholesterol, and no significant amount of inorganic constituents.

Suprarenal Glands

Gross Examination. Each suprarenal gland (Fig. 2) was approximately five times the average size, the left weighing 50 gm. and the right, 40 gm. Both were firm and of a similar appearance grossly. The cut surface presented a thin, well defined, irregular, bright yellow peripheral zone measuring up to 1 mm. in thickness. Between the ex-

* Permission was not granted to examine the contents of the cranial cavity.

ternal yellow layers, a thick chocolate-brown zone occupied the central portion of both glands. This zone measured up to 8 mm. in thickness and accounted for the bulk of the glands. In a few of the sections a thin, pale gray medulla was present in the center of the gland; it measured from 0.5 to 1 mm. in thickness.

Microscopic Examination. The greater part of each suprarenal gland was composed of a broad, relatively well defined zone of tissue bounded by the medulla internally, and by the remnants of zona fasciculata, and at times zona glomerulosa, externally. The major portion of this hyperplastic zone was made up of cell nests of various shapes and sizes which were separated by intensely dilated and engorged sinusoidal capillaries (Fig. 4). The characteristic cell in most of the hyperplastic zone was rounded or polyhedral and had an average measurement of 16 by 13 μ. Its cytoplasm was compact, granular, relatively free of vacuoles, and laden with much granular brown pigment. The cells contained one nucleus which was usually eccentric and vesicular; occasionally the nucleus was hyperchromatic. One nucleolus was the rule. The hyperemia and the brown pigment in the cells constituted the two most striking features in this zone.

The peripheral portion of the hyperplastic zone encroached on and, at times, actually replaced the zona fasciculata. It differed from the main body of the hyperplastic zone in the following respects: (1) Though numerous vascular spaces were present, hyperemia was virtually absent; (2) The component cell was larger; the average measurement was 22 by 19 μ; and (3) The cytoplasm, which frequently was compact peripherally and rarefied centrally, contained less pigment and was fluffy or finely vacuolated.

In the zona fasciculata many of the normal parallel columns of cells were replaced by the cell nests of the hyperplastic zone. The fascicles which remained, as compared with the normal gland, were considerably diminished in length, and the component cells were distinctly less vacuolated than normal.

The zona glomerulosa was slightly broader than usual; the cells were well preserved and showed no significant histologic changes. Occasionally, cells of the zona glomerulosa were seen to stream far into the subjacent zona fasciculata.

As compared with the normal cortex, there was a striking diminution in the amount of lipoid present in the cells. Although small lipoidal droplets were seen in most of the cells of the hyperplastic zone, they were especially pronounced in the remnants of the zona fasciculata. Oil red O was employed for demonstration of the lipids.

The medulla was without histopathologic alterations.

Testes

Gross Examination. The testes were similar in size and appearance (Fig. 3). They were ovoid, firm, and small, approximately two-thirds the usual size. The external surface was smooth and gray-white. Each measured 3 by 1 cm. On cut surface each was composed of a peripheral yellow-gray zone and a central, firm, well defined, dark reddish brown tumor which measured 2 by 1 cm. The tumors were lobulated and contained numerous gray streaks.

Microscopic Examination. The testicular tumors showed a similar histologic picture. They were sharply demarcated from the peripherally situated, seminiferous tubular tissue. A definite capsule was not evident. The pattern of the cell groups and the structure of the component cells were uniform. The tumors were composed of cells variously arranged in small groups and as individual elements. The cells and cell groups were separated by a rich network of collagenous tissue strands and bundles, and by innumerable capillaries, many of which were dilated and engorged with erythrocytes (Fig. 5). These engorged vessels frequently assumed the appearance of sinusoidal channels and were especially prominent at the periphery of the tumor lobules. The representative cell of the tumor was rounded or polyhedral and had an average measurement of 20 by 17 μ. Its cytoplasm, which was pale, granular, and faintly vacuolated, was characteristically condensed at the periphery of the cell and rarefied near the center. Lipids were present in the cytoplasm in only small amounts as fine droplets. In many of the cells a granular, pale brown pigment was also present in the cytoplasm. No crystalloids were seen. The nucleus of the cell was usually prominent, round, vesicular, and somewhat eccentrically located; there was usually a single, distinct nucleolus. Occasionally the nucleus was hyperchromatic. Mitotic figures were not seen. The cells of the testicular tumors bore a striking resemblance to the cells in the outer portion of the hyperplastic adrenal cortex. The vascular pattern of the tumor was quite similar to that which was present in the main body of the hyperplastic adrenal cortex.

In the tumor of the right testis was a small, discrete, almost completely encapsulated, relatively nonhyperemic, adenomatous nodule (Fig. 6). The cytoplasm of the component cells was smooth, homogeneous, and darkly eosinophilic, in contrast to the pale and granular cytoplasm of the cells occupying the tumor proper (Fig. 7). Pigment was not present in the cytoplasm. Generally the cells in this nodule were smaller than those in the main testicular tumor. The average diameters of the cells were 15 by 13 μ. Large bizarre cells with an eccentric hyperchromatic nucleus were occasionally seen. Where the connective

tissue capsule of the nodule was deficient, the cells of the nodule were continuous with the cells of the main testicular tumor. At times, cells with two nuclei, rarely more, were encountered in the main tumor and in the discrete nodule.

Tubules of the rete testis occurred throughout the tumor. They were most conspicuous at the hilar area and in the interlobular septa of the tumor. Occasional atrophic seminiferous tubules were scattered throughout the tumor.

In the periphery of the testis the seminiferous tubules were strikingly set off from the tumor. They showed a uniform, moderate atrophy. The cells were arranged in one or, at the most, two layers. Evidence of spermatogenesis was wanting. The basement membrane of the tubules was distinctly thickened. The stroma contained an increased amount of loose connective tissue. Careful search of several sections taken from different parts of the testes failed to reveal Leydig cells in the stroma of the peripherally located tubular tissue. The tunica albuginea was of average thickness.

Discussion

The morphologic identification of the testicular tumors presents the most difficult problem in the study of this case. Although tumors of the testes may originate from any one of several types of cells or tissues, it is possible, without too much difficulty, to limit the origin of the tumors in this case to either interstitial or aberrant adrenocortical cells. It is well recognized that adrenocortical and interstitial cells have many cytologic features in common, and differentiation on morphologic grounds may be difficult.

A review of the interstitial cell tumors reported in the literature [7-16] and a study of several instances of interstitial cell hyperplasia and of one interstitial cell tumor * reveal certain similarities and certain fundamental differences between interstitial cell growths and the tumors found in my case. These are tabulated as follows:

	Interstitial Cell Tumors	*Tumors in Present Case*
1. Color	Various colors have been described; usually the tumors are light in color; only occasionally are they dark brown	Dark brown
2. Bilaterality	Only one bilateral interstitial cell tumor has been reported; all others have been unilateral	Bilateral

* Dr. Paul Klemperer of the Mt. Sinai Hospital, New York City, graciously permitted me to study the slide of an interstitial cell tumor.

3. Pattern of cell growth	In large sheets, masses, or cords; never in small nests or as individual elements	Small nests and individual elements
4. Vascular pattern	Small networks of capillaries between cell groups	Innumerable vascular channels, frequently occurring as dilated and engorged sinusoidal capillaries
5. Brown pigment in cytoplasm of cells	Present	Present
6. Foamy, spongy, or vacuolated cytoplasm	May occur	Present
7. Reinke's* crystalloids in cells	Absent [12,16]	Absent
8. Leydig cells in peripherally compressed parenchymal tissue	Frequently absent	Absent
9. Precocious somatic development and hypergenitalism	May occur	Present
10. Associated hyperplasia of adrenal cortex	Never described	Present

It is apparent, therefore, that the testicular tumors in this case and interstitial cell tumors have many characteristics in common. Nevertheless, it is felt that the evidence favors an origin from adrenocortical rather than interstitial cells. This evidence is based on the fundamental similarity of the testicular tumors and the hyperplastic adrenal cortex in respect to: (1) gross appearance, (2) arrangement of the cell groups, (3) structure of the cells, and (4) vascular pattern. Additional evidence that the tumors of the testes are of adrenal rather than interstitial cell origin may be adduced from the occurrence of a small discrete nodule in the tumor of the right testis; the histologic appearance of this nodule is strongly suggestive of adrenocortical tissue (Fig. 7). It is of interest to note that in not a single reported instance of human interstitial cell tumors has there been described an associated hyperplasia of the adrenal cortex. Though the occurrence of heterotopic adrenocortical tissue in the testis proper is extremely infrequent, such instances have been recorded previously.[1,17] Adrenal rests occur more often outside the parenchymal tissue of the testis or in relation to other portions of the male genital tract.

The histogenesis of the cells in the hyperplastic adrenal cortex, as well as in the testicular tumors, is of considerable importance and interest. The hyperplastic tissue occupies the position of the zona reticu-

* Reinke, F. Beiträge zur Histologie des Menschen. Ueber Krystalloidbildungen in den interstitiellen Zellen des menschlichen Hodens. *Arch. f. mikr. Anat.*, 1896, 47, 34–44.

laris of the normal adrenal and, further, extends peripherally to encroach on and frequently replace much of the zona fasciculata. Indeed, this tissue has two characteristics in common with the normal reticularis: (1) brown pigment in the cytoplasm of the cells and (2) engorged sinusoidal channels between the cell nests. The first thought, therefore, is that the tissue represents hyperplastic zona reticularis.

In recent years the concept of a specific androgenic tissue in the adrenals, functionally and anatomically distinct from the permanent cortex or interrenal tissue, has been advanced by Grollman.[18] That these two tissues are distinct is further suggested by the embryologic studies of Keene and Hewer[19] and Uotila.[20] Under normal conditions in man, this so-called androgenic tissue, or, as it is usually termed, the fetal cortex, is abundantly present in the adrenal during fetal life. However, during the first year of extra-uterine life it almost completely disappears. At the height of its development, it is a conspicuous, intensely hyperemic zone which lies between the medulla internally and the tissue destined to become the permanent cortex externally. Grollman believed that the adrenocortical tumors and hyperplasias responsible for virilizing changes in the female, and precocious somatic and sexual development in the prepubertal male, represent a persistence and subsequent overgrowth of the androgenic tissue, rather than a proliferation of the interrenal tissue. In my case the location of the hyperplastic tissue, its pronounced vascularity, and the macrogenitosomia during the patient's childhood strongly suggest that this tissue is androgenic rather than hyperplastic zona reticularis. Unfortunately, there are at present no exact morphologic criteria for differentiating androgenic cells and cells of the zona reticularis.[21] Broster and co-workers [2] believed that the ponceau fuchsin stain is specific for androgenic cells. This stain was used, but negative results were obtained on both the adrenals and testes. Hormone assays were not carried out on the tissues, blood, or urine.

The final problem which confronts us in this case is the rapidly developing and profound peripheral circulatory failure. In the recent literature there are two reports which indicate that functional adrenocortical insufficiency may occur in association with hyperplasia of the adrenal cortex. In a case of macrogenitosomia precox reported by Wilkins *et al.*[1] there were clear-cut clinical and blood-chemical findings of adrenocortical insufficiency in the form of: (1) pigmentation of the skin and mucous membrane, (2) a craving for salt, (3) sudden death, (4) high blood nonprotein nitrogen, and (5) low blood sodium. Anatomically the adrenals showed widespread loss of normal interrenal substance and replacement of the same by hyperplastic tissue, which is

considered to be androgenic tissue. Their interpretation of these changes is that due to a loss of functioning interrenal tissue, adequate hormone was not present to sustain life. Dijkhuizen and Behr [22] reported a series of four cases of pronounced bilateral adrenal hypertrophy (cortical) in young infants, in all of whom the most prominent symptom was severe protracted vomiting followed by death. In three cases the feces were described as thin and green. In all four instances the symptoms were suggestive of intestinal stenosis. An autopsy was performed on three of the patients, and a laparotomy on the fourth. Stenosis or any other abnormality of the gastrointestinal tract could not be demonstrated. In the three cases upon which necropsies were performed, bilateral hypertrophy of the adrenals due to cortical hyperplasia was found. Although post-mortem examination was refused in the fourth case, the authors had reason to believe that adrenal hyperplasia was present likewise. Severe vomiting and other gastrointestinal disturbances are common symptoms of adrenal insufficiency in man and certain animals.[18] That lesions replacing a significant portion of the interrenal tissue, notably the zona fasciculata, may cause adrenal insufficiency is further suggested by Bennett's histochemical studies,[23] which indicate that in the cat, the cortical hormone is localized in the zona fasciculata. Since, in my case, there is adequate evidence of severe encroachment on, and actual replacement of, appreciable portions of the zona fasciculata, I think that functional adrenal insufficiency offers the most reasonable explanation for the circulatory collapse. Circulatory failure is commonly encountered in cortico-adrenal insufficiency.

It is interesting to reconstruct the possible sequence of changes which occurred in the life of this patient as the result of the adrenal and testicular lesions. His infancy was apparently normal. In early childhood the androgenic effect of the lesions became clearly manifest in the form of precocious development of the primary and secondary sexual characteristics and increased body growth. The history suggests that the excessive androgenic stimulation continued until the boy was at least 6 years of age. Beyond this age our knowledge of further somatic and sexual development is incomplete. It is noteworthy that at the age of 6 the patient's body length was 56¾ inches (144 cm.), whereas at the time of death (at the age of 20) he was only 61 inches (155 cm.) in height. It is evident, then, that minimal body growth occurred after the age of 6. Precisely what mechanism effected the relative cessation of continued somatic and sexual development after the age of 6 is not apparent. It seems probable that the epiphyses closed early in life, and this precluded the possibility of further in-

crease in height. Suggestive evidence that a relative functional cortico-adrenal insufficiency existed prior to the terminal infection came from soldiers who knew the patient well. They stated that he tired from physical activity more easily than his associates; the tiredness at times was extreme. Recent studies [24] indicate that hormones of the adrenal cortex play a significant rôle when the organism encounters or is subjected to situations of stress. The adrenocortical insufficient state in man as well as in experimental animals is characterized by weakness, exhaustion, and ultimately by collapse. Further, fatigue is accompanied by an increased urinary output of the 17-ketosteroids, the primary source of which is thought to be the adrenal cortex.[24] It seems reasonable to assume, therefore, that the patient had enough functioning interrenal tissue to meet ordinary needs. When the demands became excessive (physical exertion, severe infection), inadequate amounts of cortical hormone were available, and a state of insufficiency developed.

Summary

A case is reported of pronounced bilateral hyperplasia of the adrenal cortex associated with bilateral testicular tumors in an adult male. In childhood the patient exhibited precocious genital and somatic development, whereas in adult life he showed no apparent abnormalities except for small stature. He died in the course of pneumonia (pneumococcus, type 1), middle lobe, right lung. One of the outstanding features of the clinical course of the infection was a rapidly developing and severe circulatory collapse. Histologic study suggests that the testicular tumors were derived from aberrant adrenocortical tissue rather than from interstitial cells. It is thought that peripheral circulatory failure was probably due to cortico-adrenal insufficiency.

I wish to express my gratitude to Dr. Carrol M. Pounders of Oklahoma City, Oklahoma, and to Mt. Sinai Hospital, Cleveland, Ohio, for providing clinical notes; and to the resident civilian consultants and to the regular staff of the Army Medical Museum for their advice and assistance in the study of this case.

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[*Illustrations follow*]

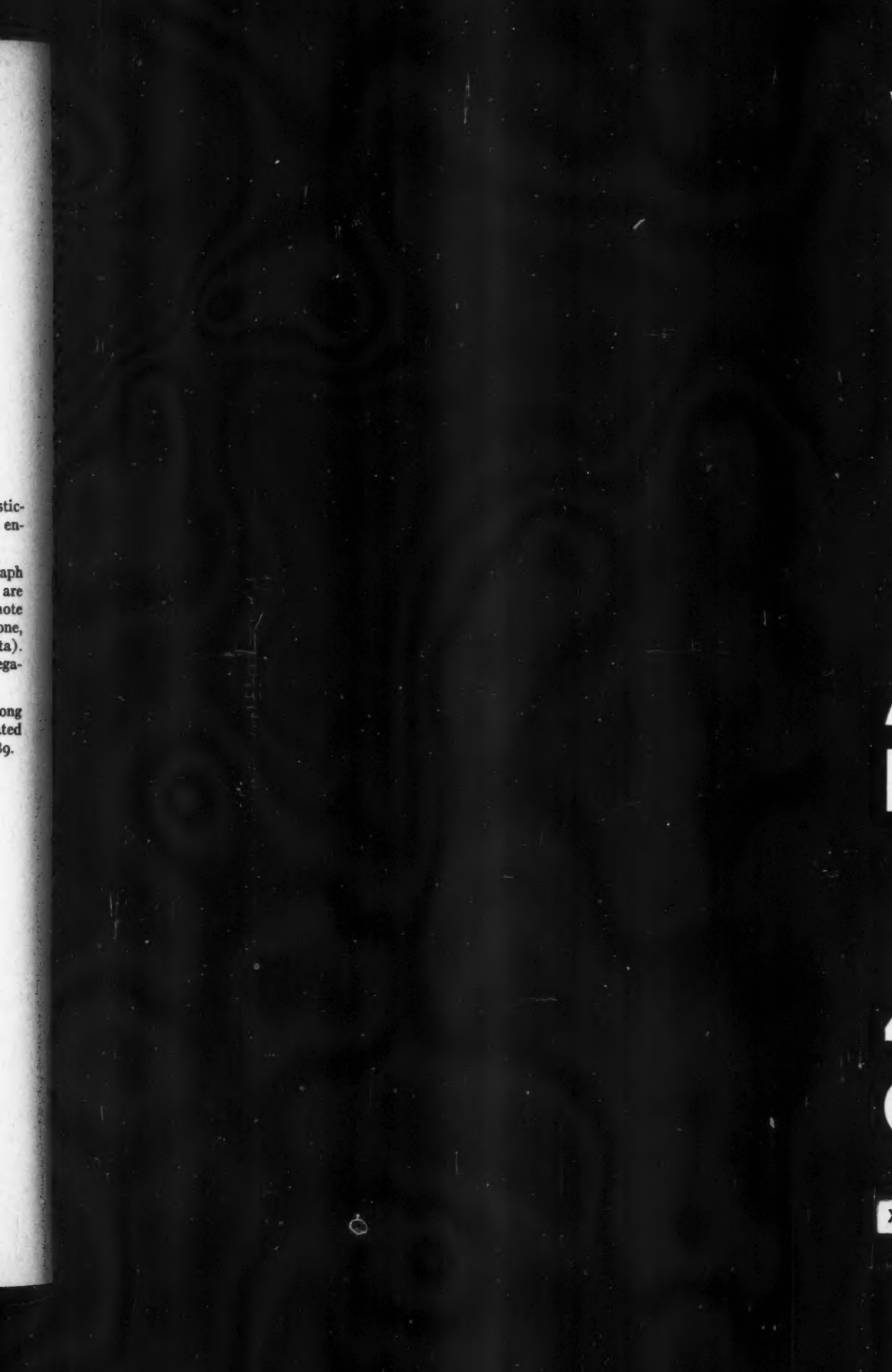
DESCRIPTION OF PLATES

PLATE 33

FIG. 1. A case of hyperplasia of the adrenal cortex associated with bilateral testicular tumors. Photograph of patient at 29 months of age. The penis is enlarged. The pubic hair cannot be seen.

FIG. 2. Gross appearance of suprarenal glands. In the upper part of the photograph is the cross section of a normal suprarenal gland for comparison. Below it are two representative cross sections of the suprarenal glands in this case. Of note are the pronounced enlargement of the glands, the dark inner hyperplastic zone, and the encroachment of the latter on the peripheral yellow zone (fasciculata). The medulla is not present in either of these sections. × 1½. A.M.M. negative no. 74988.

FIG. 3. Gross appearance of left testis. The testis has been bisected through its long axis, exposing both cut surfaces. The central dark tumor is sharply demarcated from the pale, peripheral, testicular tissue. × 2. A.M.M. negative no. 74989.



1

2

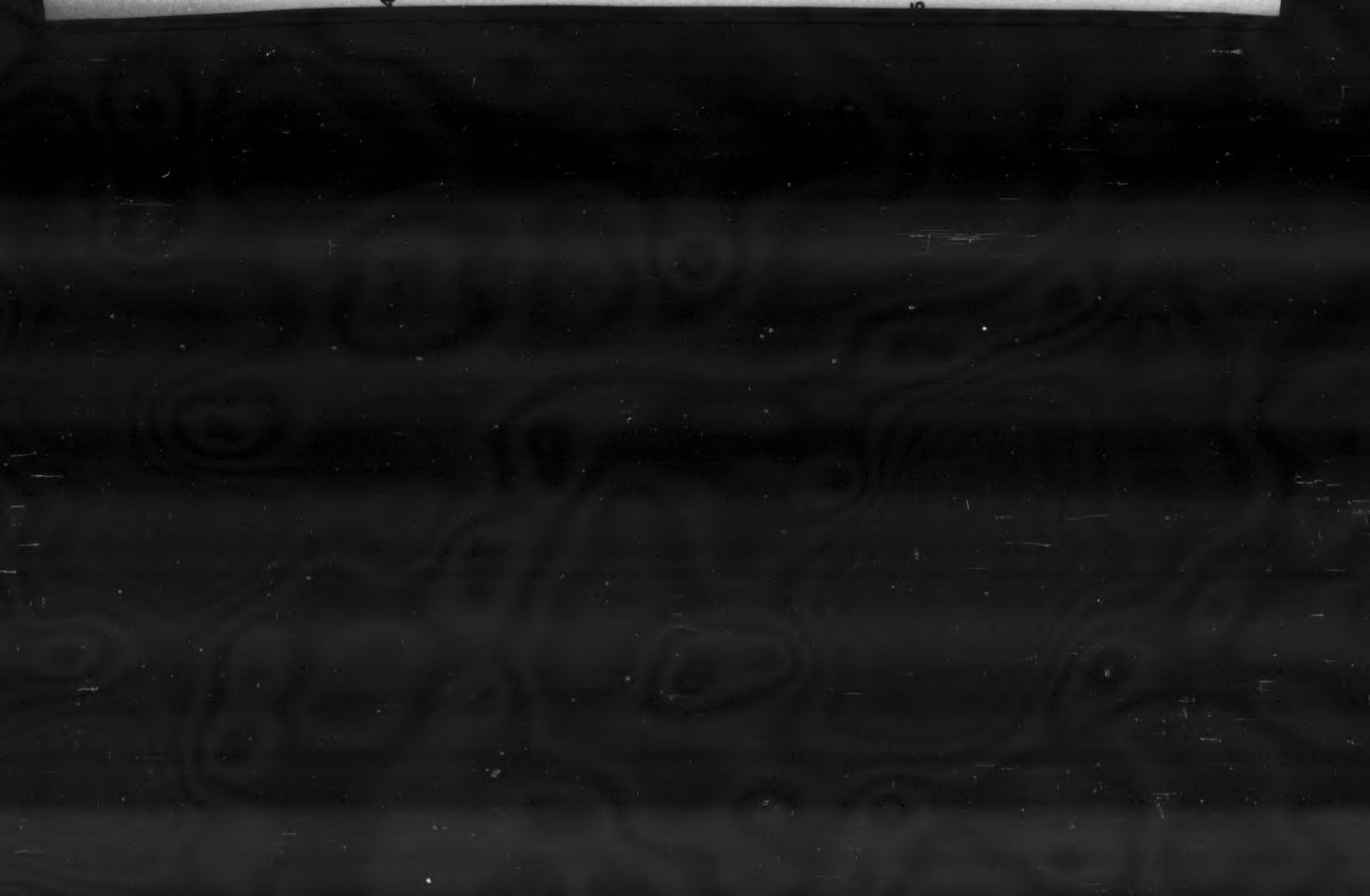
3

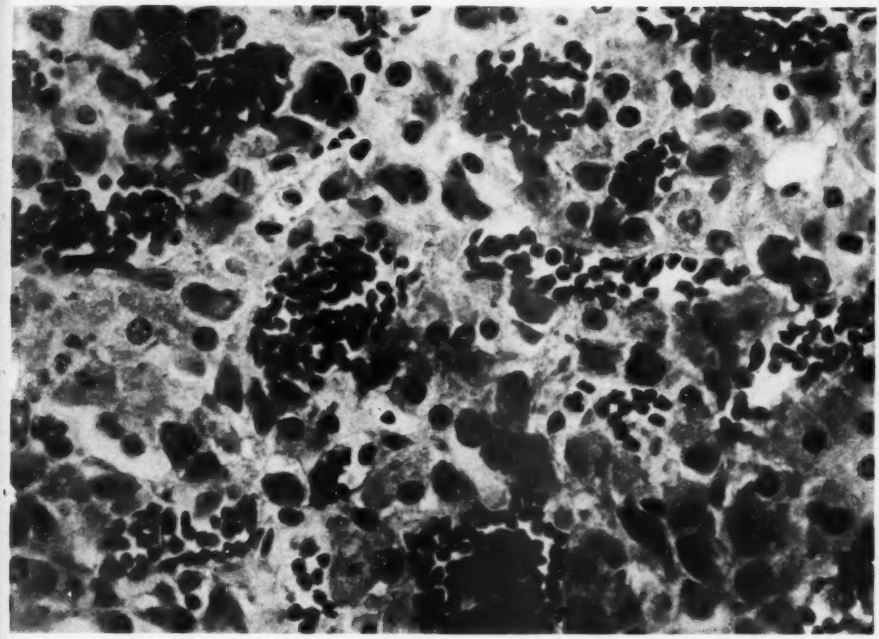
Cohen Adrenal Hyperplasia with Testicular Tumors

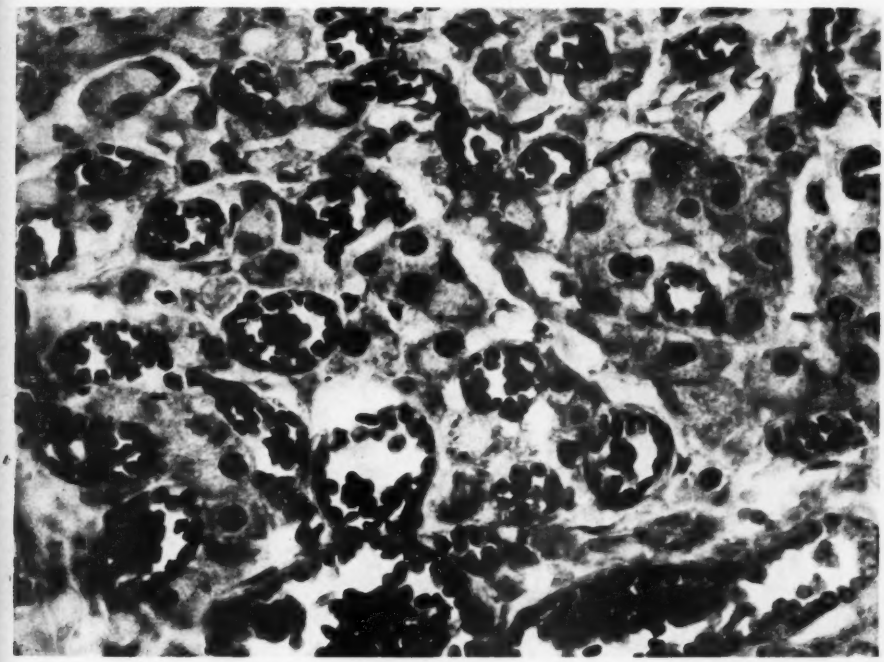
PLATE 34

FIG. 4. Suprarenal gland showing inner portion of hyperplastic zone. Of note are the dilated and engorged sinusoidal capillaries and the irregular groups of cells between them. × 515. A.M.M. negative no. 77624.

FIG. 5. Testicular tumor, showing the similarity of the vascular pattern and of the arrangement of the cell nests to the hyperplastic adrenal cortex. In the testicular tumor the cells are slightly larger and the cytoplasm more granular than in the inner portion of the hyperplastic zone of the adrenal cortex shown in Figure 4. The peripheral condensation and central rarefaction of the cytoplasm are evident. The blood vessels have prominent endothelial cells. × 515. A.M.M. negative 77553.







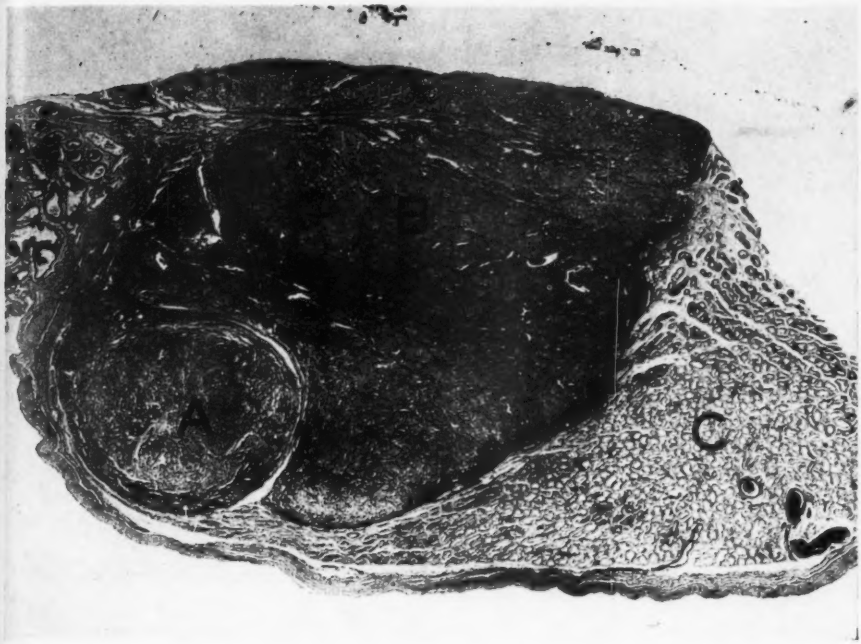
Cohen

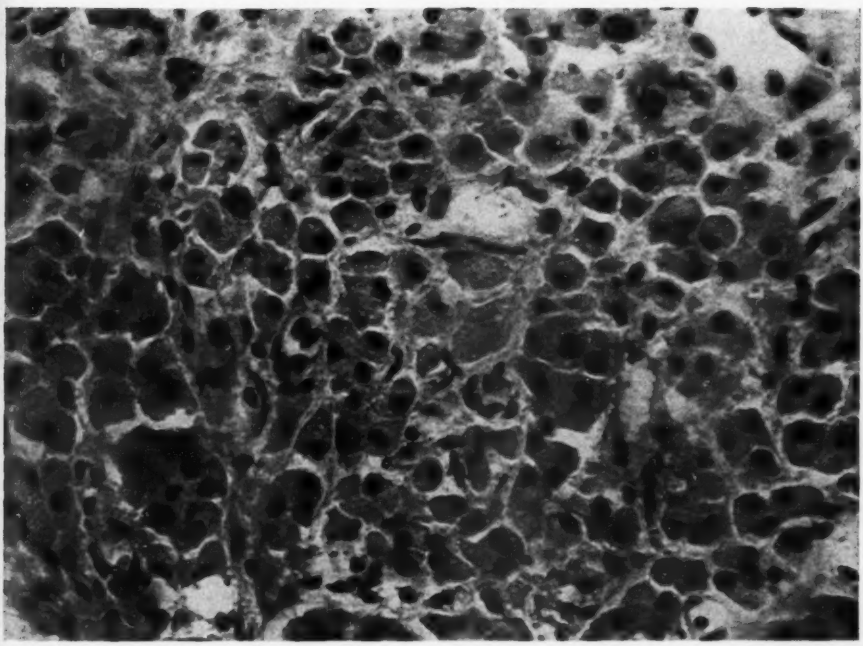
Adrenal Hyperplasia with Testicular Tumors

PLATE 35

FIG. 6. Small, sharply circumscribed adenomatous nodule of adrenocortical tissue located within main tumor of right testis. (A) Discrete nodule. (B) Main tumor. (C) Atrophic parenchyma of testis. × 10. A.M.M. negative no. 77621.

FIG. 7. High-power view of discrete nodule shown in Figure 6. The relative avascularity of the nodule and the similarity of the component cells to adrenocortical cells are apparent. × 515. A.M.M. negative 75793.





Cohen Adrenal Hyperplasia with Testicular Tumors

STUDIES ON AMEBOID MOTION AND SECRETION OF MOTOR END-PLATES

VII. Experimental Pathology of the Secretory Mechanism of Motor End-Plates in Thermal Shock *

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In reference to the shock of heat stroke, Moon [1] made the following statements (page 366): "The mechanism by which heat stroke causes circulatory death is not known, but many believe it to be through effects upon the central nervous system. Neither is it known whether disturbances in the central nervous system, either organic or functional, affect the circulation through the sympathetic system or through some other route. But it is evident that the same cycle [in the production of shock], whose effects have been seen in numerous other conditions, is operative here."

After years of study of the problem of shock, Cannon [2] clearly stated the unsolved problem of the onset of shock as follows (pages 140 and xi, respectively): "The mode of action of the initiating agent in secondary shock is thus left unsatisfactorily explained." "The reader should understand from the beginning that the mystery of the onset of shock has not been definitely cleared away despite a considerable increase in our knowledge of it, and that there still remains much work to be done before we shall have elucidated all the factors which play a rôle in its establishment." This is particularly true of the rôle of the pathologic motor end-plates in both primary and secondary shock. Cannon and his colleagues did not use morphologic methods in the study of shock. Moon and his associates did employ anatomic methods but did not study the changes in the motor end-plates.

The present study is an attempt to reveal, by the methods of experimental morphology, gold impregnation, and teasing of the innervation of muscle tissue, (1) the nature of the normal mechanism of secretion in motor end-plates of skeletal muscle; and (2) the rôle of this exaggerated secretion in the structural exhaustion of the motor end-plates and alteration of muscle structure and function in the pathologic mechanism of shock produced by heat.

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Materials and Methods

The preconception of the histologists, that there is a well defined and constant line of demarcation between nerve and muscle, determined the selection of histologic technics employed in the past in the study of the neuromuscular apparatus. The gold impregnation and teasing method had been replaced by some histologists for those in which silver and methylene blue were used. The granules of Kühne were poorly portrayed by silver and methylene blue but were clearly defined with gold. When there was variation in the intensity of impregnation of the axons and granules with the uncontrolled gold solutions, the method was discarded as unreliable. The fact that impregnation with gold and teasing whole mounts of muscle fibers revealed both the variations in the total morphologic structure and variations of intensity of impregnation of motor end-plates stamped this method as superior to any other. When normal and abnormal tissues were subjected simultaneously to the same solutions, the variations were clearly visualized and could be evaluated.

These changes in form and quantity of nervous substances were, therefore, intrinsic to the tissues and were not due to extrinsic variables in the gold technic. The apparent unreliability and real variations of results misled histologists, and resulted in their discarding a technic that contained the clue to the function of the motor end-plates. Technicians were hunting for a standardized result. The motor end-plates could not be standardized with a constant and fixed structural pattern any more than the cross striations of muscle could be arbitrarily classified with a fixed number of constant sarcomeres. The tissues may be overstained or understained with gold, but under controlled conditions this is avoided, or may be detected and evaluated.

The minute differences of structure and the variable affinity of the neuromuscular apparatus for gold used under the conditions in which comparisons were made, revealed these important facts about the motor end-plate: (1) The pleomorphism is due to ameboid motion; (2) The quantitative variation of granules is due to a secretory mechanism by which the terminal axons of the end-plate are attenuated or depleted by their direct transformation into granules discharged into the myoplasm of the muscle fiber. It was an error, therefore, to consider the junction of nerve and muscle as possessing a fixed structure when, in reality, the relationship was one of elusive continuity rather than that of a deceptive discontinuity. It was no less an error to discard the best method available because of variations of morphologic results, which portrayed the secretory mechanism of the motor end-plates.

The revelation of the gland-like nature of the structure of the motor end-plates is dependent upon the methods selected to demonstrate the polarity of the secretory mechanism of the neuromuscular apparatus. There are several methods commonly used for the microscopic study of the motor end-plates, *viz.*, the silver, the methylene blue, and the gold method. The authors of current histologic textbooks and manuals of technic claim that the gold method is uncertain and unreliable. There is considerable variation of opinion as to which method is most accurate. Experience counts for much in accuracy. The gold method may be easily mastered: our freshmen medical students use it with good effect as a routine laboratory procedure. Impregnation with silver and *intra vitam* staining with methylene blue do not visualize the granules of Kühne as well as does impregnation with gold chloride. Attempts to study the end-plates in living muscle are now in process in our laboratories. This is a difficult problem because of the similarity of the refractive index of living nerve endings and muscle. There appears to be a more specific affinity of the axoplasm and its discharged granules for gold than for any other substance used thus far in the staining or impregnation of the end-plates. *In vitro*, gold chloride forms a specific precipitate with both choline and acetylcholine.

Teasing is better than sectioning for the preservation of the polarity of the secretory mechanism of the motor end-plates. By the teasing method significant changes in the epilemmal axon, hypolemmal ramifications, granules of Kühne, and in the cross striations of muscle may be observed as a whole, simultaneously and in relationship one to another. This structural relationship may be photographed. This total anatomic relationship of nerve and muscle is obscured, if not destroyed, by sectioning the muscle. Gold impregnation and muscle teasing are old methods and therefore are discredited in the minds of a few investigators who possess inadequate experience with them. For the purposes of this study, nevertheless, these methods are superior to any other neurologic technic. The nuclei may be counterstained. The Bielschowsky [3] and Boeke [4] silver and sectioning methods are good for clear visualization of the nuclei and of the variable neurofibrils formed by the streamlining of protoplasmic flow in the axis cylinder. These methods were used as a check against the gold chloride method of impregnation and teasing of muscle. The Spielmeyer technic was used to detect demyelination of the peripheral innervation of muscle and staining with scharlach R for acute fatty degeneration of nerve fibers.

White rats (*Mus norvegicus*) were subjected to shock due to scalding by the method of Prinzmetal, Hechter, Margoles, and Feigen.[5] This method possessed the following features: the degree of trauma

was objectively controlled and reproducible; the method was simple and fast, so that a sufficient number of animals yielded results of statistical significance; finally, the shock-producing procedure resembled a type of trauma which produces shock in man.

The method consisted in immersing the entire body surface, except the head and neck, of either etherized or nonetherized rats, for definite periods of time in a water bath set at different water temperatures. The degree of trauma was a function of the duration of exposure and of the temperature of the bath.

Two hundred rats, average weight 200 gm., were subjected to immersion for 10 seconds at 75° C. The animals were not allowed access to food or water after immersion. At 30-minute intervals the gastrocnemius muscles were excised from 40 of the most prostrated animals, prepared by the gold chloride technic,[6] and teased. Preparations were stained for the study of the myelin sheaths of the epilemmal axons.

Fifty rats were subjected to a temperature of 90° C. The mean survival time was 75 seconds. After immersion 10 of the most prostrated animals were selected at 15-second intervals and their gastrocnemius muscles were excised. The muscles were immediately subjected to the gold technic.

At different time-intervals after the rats were immersed at 75° C. for 10 seconds the temperature of the skin decreased and hemoconcentration was evident, as well as dyspnea. Great muscular weakness and flaccid paralysis were present in later stages. Rats subjected to 90° C. for 10 seconds became spastic at once and exhibited extensor rigidity preceded by muscular twitchings. This pathologic functional response of immediate spasticity of the muscles was exhibited, likewise, by 20 rats immersed in water at 90° C. for 1 second. The temperature of the muscle of these animals varied from 37.8° to 42.4° C.

Twenty rats, average weight 200 gm., were used as controls. The gastrocnemius muscles were excised 15 minutes after the intraperitoneal injection of nembutal, 4 mg. per kg. These relatively normal control muscles were run through the same solutions concurrently for the same periods of time as those muscles excised from the scalded animals. The histologic variations of the motor end-plates are due, therefore, to factors intrinsic to the muscle, and not to uncontrolled accidental variations of technic extrinsic to the muscle. The simple and easily reproducible experiments under controlled conditions herein outlined may be used as classroom demonstrations of the secretory mechanism of the motor end-plates in skeletal muscle.

Since photographic evidence is the only acceptable means of over-

coming the current confusion about the true structure of the motor end-plates in shock, an apparent abundance of illustrations is presented. On the other hand, all that has been written on this subject for over a century has convinced no one. This photographic series constitutes objective evidence which any one may easily observe and as easily evaluate. Thus a permanent atlas is at hand to serve anyone as a point of departure for future experiments on the pleomorphism of the neuromuscular apparatus.

Small variations from the normal, standard intensity of the impregnation with gold may be attributed by some critics to faulty technic of overstaining or understaining and not to any irregularity in the quantity and structure of the chrysophilic axonic substances in the end-plate itself. Since normal and abnormal tissues were subjected concurrently to the same concentrations of solutions and for the same periods of time, these differences are due to variations in the quantity of the axonic substances in the motor end-plates themselves. These minute differences in the quantity of Kühne's granules, and variations in the form and structure of the hypolemmal axons of the motor end-plates, offer real footholds for scientific advance in the anatomy, physiology, and pathology of the neuromuscular apparatus.

Experimental Observations

I. The Pathologic Physiology of Muscle in Thermal Shock

There were variations not only in the time of onset but also in the type of the convulsions leading to muscular rigor in primary thermal shock. Certain animals had typical, severe, clonic spasms characterized by extensor rigidity. Some animals were thrown into a single, violent, tonic convulsion and remained in this condition. When rats were immersed for 1 second at 90° C., the convulsive movements frequently began with twitching and then vigorous shaking or shivering of the body. The typical clonic convulsion was followed by tetanic spasms of extensor rigidity of the entire body that preceded the death of the animal. Some animals experienced dyspnea and gasped for air during the time just prior to the clonic convulsion. Respirations were at first rapid, 150 to 200 per minute, and either gradually or suddenly became progressively slower until they reached 15 to 35 per minute.

The short time-exposures, by dipping the animals (except the head) for 1 to 10 seconds in water 75 to 90° C., were insufficient for the direct effect of heat to be transmitted to muscle by conduction. Some heat may have been transmitted through the blood stream. But when the skin was in contact with water at a temperature of 75° to 90° C., the

underlying gastrocnemius muscle in 24 animals had a temperature that varied from 37.8° to 42.4° C. The muscle rigor, therefore, was not the direct effect of heat on muscle producing heat rigor by protein coagulation. The clonic and tonic convulsions or cramps of the muscles were produced by reflex nervous action on the neuromuscular apparatus, arising from the initial sensory impulses generated by the caloric stimuli on the skin surface, reflected from the spinal cord through the motor innervation. The violent motor impulses transmitted to the neuromuscular apparatus were coincident in time with a massive transmission of axonic material into the end-plate and a torrential flow of the hypersecreted nervous substance away from the end-plate out into and between the muscle fibers. There was a corresponding and coincidental decrease in the diameters of the axons of the medullated branches of the sciatic nerve to the gastrocnemius muscle.

At autopsy the subcutaneous vessels were found enlarged, hyperemic, and densely packed with clumps of coagulated red blood cells. The intramuscular capillaries and venules, likewise, were enlarged in many places and in a condition of active hyperemia in those animals that were in thermal shock for 1 to 8 hours. Perivascular edema and packing of the minute vessels of the muscle with red blood cells were late manifestations of shock by heat. The muscles of those animals dissected within 10 to 30 seconds after the primary heat stimulus to the skin had the explosive pathologic changes in the motor end-plates, and not in the capillary bed of the muscles. The muscles in rigor produced by the reflex action of heat frequently did not respond to either direct or indirect mechanical or electric stimulation, but there was individual variation in the response. Many muscles in strong tonic contraction or tetanic spasm induced by the reflex action of heat did not manifest irritable retraction when transversely severed. Such muscles frequently had extensive axonorrhea or a discharge of the axonic nervous substance in the muscle. Many of the spastic muscle fibers were disorganized and in a condition of acute granular, hyaline, or Zenker's degeneration. During the late stages of thermal shock the muscles were frequently in a condition of flaccid paralysis. Such muscles had a dissolution of many of the motor end-plates and the nerve fibers terminating in them. There was a loss of the gold-staining substance in the motor end-plates that extended progressively in a centripetal direction in the epilemmal axons. This resulted in a local loss of many motor innervations of muscle fibers at the peripheral myoneural junction, and flaccid paralysis or muscle weakness. There was good correlation between abnormal structure and function of the neuromuscular appara-

tus. In the early stages of spastic palsy reflexly induced by heat, there was extensive secretion of nervous substance manifested by the gold-staining axonic material discharged into the muscle. In the later stages of thermal shock the animals were frequently in a condition of flaccid palsy. Many muscles from such animals had a structural loss of innervation at the junction between nerve and muscle. The pathologic function manifested by the skeletal muscle, therefore, was highly variable and was dependent upon the strength and duration of the stimulus, upon the time interval after onset of the stimulus, and upon the individual resistance of the animals to thermal shock.

Muscles reflexly manifesting a state of tonic spasm, rigor, or cramp due to the application of heat superficially applied to the end-organs of sensory nerves in the skin, presented two important problems: (1) Was the tonic spasm of muscle, induced by the nervous reflex to heat applied to the skin, caused solely by the transmission of immaterial trains of waves of electric energy constituting the nervous impulse to muscle? If so, morphologic methods are impotent to answer this question except by indirect evidence detected in alterations of the structure of the cross striations.[7] (2) Was the tonic spasm of muscle, induced by the nervous reflex to heat applied to the skin, associated in time with the transmission of a real material substance from nerve to muscle? If so, then morphologic methods are competent to reveal histologically this nervous substance discharged from nerve endings into muscle. The morphologic evidence herein presented demonstrates conclusively that there is a substantial transfer of nervous material from the nerve ending to muscle associated with functional activity.

The gross manifestations following shock produced by heat were comparable to those previously described for the viscera. The blood vessels of the gastrointestinal tract were pale, collapsed, and bloodless. The liver had blood vessels packed with red blood cells. Upon sectioning the liver, there was little or no bleeding. The spleen was retracted and failed to bleed upon sectioning, soon after trauma by heat. The kidneys and lungs had active hyperemia and vessels packed with red blood cells. These vascular changes became prominent during the late stages. Muscles were more red than normal ones and they were more friable and softer to teasing. The accumulation of metabolites evidently changed the consistency and elasticity of the fibers. Some paralyzed muscles failed to respond to transection of the spinal cord within 60 seconds after thermal trauma, as well as to direct mechanical cutting, and to direct and indirect stimulation by the galvanic and faradic currents. The rectus abdominis muscle demonstrated well the reflex

stimulation due to thermal shock to the skin by increased visibility of its contours: the lineae alba, semilunaris, and inscriptiones.

There was manifested the so-called bloody tears or chromodachryorrhea in 10 per cent of the rats at various time-intervals after immersion at 75° C. for 10 seconds. This biologic response has been considered the effect of increased amounts of acetylcholine or dacryorrhetin in the circulating blood stream.

II. The Pleomorphism of the Normal Motor End-Plates Compared with that Produced by Magnesium Sulfate and Strychnine

At the entrance of the axis cylinder of the medullated nerve fiber into the muscle fiber, the sheath of Schwann joins with the sarcolemma and the nerve loses its medullary sheath, but the axis cylinder passes into the muscle. The axon, upon entering the muscle fiber, divides dichotomously into a number of ramifications. The nerve plates (*terminaisons en plaque*) have two parts: (1) the ramifications of the axis cylinder, and (2) the variable amount of finely granular substance or sole plate of Kühne. This granular sole is abundant in the dark, retracted endings and is diminished in amount or completely depleted in the light, expanded plates. The axons of the terminal plate divide into many branches resulting in a variety of shapes. There are irregular dilatations and constrictions of the branches, like a string of beads. In the plate-like endings these ramifications are immediately under the sarcolemma. The granular sole is or is not in immediate continuity with the axonic divisions. The nuclei were divided by Ranvier [8] into three groups: (1) nuclei of the granular substance (*noyaux fondamentaux*); (2) nuclei belonging to the ramifications of the nerve (*noyaux de l'arborisation*); and (3) nuclei of the sheath of Schwann covering the terminal axons (*noyaux vaginaux*). It was impossible by morphologic criteria or differential staining to determine whether these nuclei are muscular or nervous in origin. The presumptive evidence was strong that the nuclei of the sole of Kühne were clusters of nuclei of the muscle fiber.

Where the epilemmal axon becomes continuous with the terminal plate-like expansion it divides and extends with further ramification, and thereby greatly increases its secretory surface area. The granular sole of Kühne is formed by the secretion granules of the discharging tips of the divisions of the axon. There is a direct transformation of the terminal axons in muscle into the granular sole of Kühne. The granules are poured out by the axons in arborescent arrangement and become incorporated into the myoplasm of the muscle fiber. Before

1874, Frey[9] (p. 323) made the following pertinent remarks, which have been ignored in recent times: "Now, if, as would appear to be the case, the distribution of the nerve fibre be confined to the immediate mass of the terminal plate, the extremities of the muscle fibre must remain without nervous supply, in that the former is set into the latter at about its middle. But the fleshy matter manifests contractility at the extremities also!"

The intramuscular axons, by progressive diffusion of the secreted granules, become merged throughout the fleshy mass of the muscle fiber. There is, therefore, a real anatomic blending of nerve and muscle by the periodic discharge of nervous material into the myoplasm. By the secretory mechanism of the motor end-plates there is anatomic and chemical continuity, and not discontinuity, as now taught, between nerve and muscle. There is a real, substantial fusion of nerve and muscle through the products of secretion of the nerve endings into muscle. There is, therefore, no constant line of demarcation between nerve and muscle. A search for it is like an attempt to discover a constant location where fresh water ends and salt water begins in the confluence between the Columbia River and the Pacific Ocean. This change depends upon many conditions, *viz.*, rate and quantity of flow, tide, and rate of diffusion.

The concept of Jordan and Speidel[10] that muscle has a structure composed of static membranes called sarcomeres, fixed in number, was an old one which had befuddled investigators of muscle structure for over a century. The changeable form of both motor end-plates and related cross striations invalidated any concept of constancy of structure, or fixation in number, of sarcomeres. The structure of the neuromuscular apparatus was highly variable corresponding to the periodic discharges of nervous substances into muscle. With each discharge the nervous substances blended with the myoplasm and there were concomitant changes of muscle structure as well as of the motor end-plates. The periodic discharges of nervous substances into muscle constituted one factor that determined the variation of internal structure of muscle.

Cannon[11] asked what standard we used to control our experiments in order to evaluate the normal from the pathologic changes in the motor end-plates. This was a pertinent question for the reason that characteristic changes were obtained by different local and general anesthetic agents, states of nutrition, and mechanical means employed prior to excising the muscle for microscopic study. The so-called normal had wide limits of variation. The motor end-plates quickly dis-

appeared during rigor mortis. Because of the normal sensitivity and pleomorphism of the motor end-plates, the problem was comparable to the study of the various complex forms of the waves that compose breakers along a diversified coastline. In this study we designated as standard or relatively normal (Figs. 1, 6, 23, 24, 25, and 27 to 32) those variations of form observed in the neuromuscular apparatus from muscles excised 15 minutes after the intraperitoneal injection of nembutal, 4 mg. per kg.

The relatively normal trees (Figs. 1 and 6) of motor end-plates have 10 to 20 per cent of the motor end-plates in a state of retraction and 80 to 90 per cent in a state of expansion in a differential count of 5,000 motor nerve endings, in 20 gastrocnemius muscles excised from 10 rats. The normal retracted endings surrounded by the granular sole varied from 20 to 40 μ in length. The expanded end-plates with a decreased quantity of the granular sole varied from 40 to 70 μ in length.

The cross striations were finer, closer together, and increased in number in relation to the expanded rather than to the retracted motor end-plates. If the motor end-plates did not influence the cross striations of muscle (and these cross striations are constant structures according to Jordan's [12] "sine qua non" for identification) the following questions are pertinent:

1. If the sarcomeres are constant in number in relation to each motor end-plate and in each muscle fiber, why do the expanded plates have finer striations, and more of the closely spaced striations, than are related to the retracted end-plates?

2. If the sarcomeres are constant in number in relation to each motor end-plate, would one not expect the striations to be spread farther apart in relation to the expanded plates and to lie closer together in relation to the retracted plates? The reverse relationship, however, was found.

3. If the sarcomeres are constant in number in a muscle fiber, how are they maintained when the discharge of nerve substance in muscle blends with the myoplasm?

4. Why are the cross striations frequently absent in relation to some motor end-plates?

There was, therefore, sufficient reason for the inclusion of those changes of end-plates excised from muscles in a state of flaccid paralysis 2 hours after the intraperitoneal injection of magnesium sulfate (Fig. 4), 2 gm. per kg. The retracted end-plates composed 25 to 35 per cent of the total of 5,000 end-plates counted in 20 gastrocnemius muscles from 10 animals. This proportion of retracted motor end-

plates (Fig. 4) was in contrast to that (Fig. 5) of expanded end-plates in the rigorous gastrocnemius muscle excised during an initial convulsion 10 minutes after the intraperitoneal injection of 0.5 cc. of 1:1000 strychnine sulfate. The expanded end-plates, with a diminution or complete depletion of the granular sole of Kühne, composed 95 to 98 per cent of 5,000 end-plates counted in 20 gastrocnemius muscles from 10 animals. There were numerous pseudopod-like, elongated branches in the motor end-plates during the early stage of expansion caused by strychnine.

This neoformative influence upon the ramifications and increase of surface area by ameboid expansion of the motor end-plates favored the secretory discharge of a fine spray of neuronic granules into muscle during the early period following strychnine stimulation. The chemical effect of strychnine stimulation had a morphologic expression in the structural changes of the motor end-plates and cross striations in the skeletal muscle fibers. The clear-cut photographic evidence of pleomorphism of the neuromuscular apparatus after nembutal, magnesium sulfate, or strychnine sulfate served as relatively normal and abnormal variations of changes of structure and as a base line of reference for evaluating the deviations produced by thermal shock.

The dark, anisotropic cross striations were coarser, and were separated by wider, light, isotropic bands in the flaccid muscle (Fig. 4) excised 2 hours after the injection of magnesium sulfate, than were those after the use of strychnine (Fig. 5). The latter were finer and more closely spaced than the former. This change of pattern was not due to the simple mechanical approximation and remotion of a constant number of sarcomeres in a "shuttle-like shift," like the opening and closing of the constant folds of an accordion. These changes in the cross striations were the structural expressions of changes in mechanical energy and chemical composition and concentration which are part of the ceaseless reactions of metabolism that underlie the variable tonicity, heat production, and mechanical activity of the muscular furnaces and internal combustion engines of motion of the living organism.

III. The Histopathology of the Neuromuscular Apparatus in Thermal Shock

1. *Achrysophilia and Hypochrysophilia of Motor End-Plates.* Muscles excised from rats 2 hours or more after immersion in water, except the head and neck, 75° C. for 10 seconds, had the majority of the motor end-plates (3,765 of 5,000 counted) completely liquefied (Figs.

3, 49, 68, 69, 70, 72, and 74). This final result leading to explosive disappearance of the motor endings was likewise related in many places (Figs. 68 to 70, and 74) to an acute granular degeneration and hyalinization of the cross striations of the muscle fiber. These changes of the hypolemmal axons of the motor end-plates were likewise accompanied by different degrees of depletion ending in complete exhaustion (Fig. 74), achrysophilia, and hypochrysophilia, of the substance with an affinity for gold in the epilemmal axons.

The liquefaction of the motor endings resulted in structural loss of innervation at the neural junction. Progressive hypochrysophilia to achrysophilia were those final conditions of exhaustion in which the axonic materials of the motor end-plate were discharged and dispersed in the myoplasm.

In some locations (Figs. 68 to 70), where the motor end-plates had disappeared, the clusters of dark, rounded, pyknotic nuclei, 6 to 15 in number, were clearly revealed by counterstaining with hemalum after gold impregnation. These nuclei of the depleted sole of Kühne appeared to belong to the myoplasm. They frequently were made visible without a counterstain when lactic acid was injected locally in the muscle. Lactic acid caused a rapid disappearance of the motor end-plates and paralysis due to the peripheral local loss of innervation in the muscle.

2. *Hyperchrysophilia of the Motor End-Plates.* The first morphologic result of heat applied to the skin was reflexly produced by an augmentation and overflow of the quantity of substance in the motor end-plates which had an affinity for gold (Figs. 2, 7, and 50 to 62). This increased blackening of the motor end-plates was not due to overstaining. The normal and abnormal muscles were run through the same technical procedures at the same time. This increased affinity of the end-plates for gold, due to the transmission of increased amounts of substances from the epilemmal into the hypolemmal axons, was accompanied by variations in the structure of the axons proximad to the plate. There were variations in the size of the swellings and constrictions in the moniliform patterns of the epilemmal axons. The changes in structure observed were compatible with increased conduction distally of nerve substances into the terminal axons of the motor end-plates.

3. *Axonorrhea of the Motor End-Plates.* The second result of the reflex activity of heat applied to the skin was an abnormal discharge and projection (Figs. 8 to 22, and 50 to 67) of the nervous axonic substances into the myoplasm of the muscle fiber.

The best time to observe this short-lived phase in the secretory cycle

of the motor end-plates was within 10 to 30 seconds after the rat was immersed in water, except the head and neck, for 1 to 10 seconds at 90° C. The gastrocnemius muscle was in a reflex state of spasm or rigor and had a muscle temperature of 37.8° to 42.4° C. The initial effect of heat applied to the skin for short durations on the form of the motor end-plates was via the nervous reflex arc and not by direct conduction from skin to muscle. The muscle was excised immediately and processed at once for gold impregnation. This period in motor end-plate discharge was both tenuous and ephemeral. The quantity of discharge was more copious after reflex action due to heat stimulation (Figs. 2, 7, and 50 to 62) of sensory nerves in the skin, however, than it was in the normal (Figs. 1, 6, 23, 24, 25, 27 to 38, 71 and 73) controls.

This discharged axonic material in the muscle fiber was either densely opaque with gold impregnation or cross-striated. Its cross striations were or were not in alignment with those of the muscle fiber. There were various stages in the projection, diffusion, and dissolution of the neuronic substances secreted into the myoplasm of the muscle fiber. The cross striations of the muscle offered no obstacle to the projection and diffusion of the axonic substances discharged from the motor end-plates. These cross striations, therefore, were not rigid membranes or hard and fast partitions. The easy projection of axonic substances and streamlining of the cross striations were morphologic evidences that render untenable the theory that the muscle fiber is divided into a constant number of compartments or sarcomeres by rigid partitions. There was good correlation of abnormal muscle structure and function by the morphologic presence of the nervous discharge of inclusion masses of Kühne's granules into the myoplasm and the spasm of muscle reflexly induced by heat.

The discharged nervous secretion into the myoplasm had an increased affinity (hyperchrysophilia) for gold and was in continuous relationship with the motor end-plate or projected as discrete masses throughout the muscle fiber. The arrangement of the discharged secretion in juxtaposition to the motor end-plate was unipolar (Figs. 50 to 55), bipolar (Figs. 58 and 59), or multipolar (Figs. 8, 10, 61, and 62). The form of the nervous substance secreted into the myoplasm was likewise variable, namely: (1) as discrete granules (Figs. 12 and 63); (2) as vacuoles (Fig. 64); (3) as short or long fusiform and irregular masses (Figs. 65 to 67); and (4) as short or long, Indian-club or arrow-shaped projections (Figs. 10 and 11). The axonic masses projected away from the nerve terminal were usually within the myoplasm but some were found external to the sarcolemma of the muscle

fiber. In such conditions the motor end-plates were missing and the epilemmal axons terminated external to the sarcolemma of the muscle fiber.

The axonic material descended from the epilemmal axons into the hypolemmal axons and discharged between and away from the branches of the end-plate. This discharged secretion formed either a small (Fig. 40) or a large hub (Fig. 41) in the end-plate. This central mass of Kühne's granules formed, at times, a ring with a thick rim (Fig. 42) from which the mass or masses of discharged nervous substance extended into the myoplasm. The hypolemmal axons of the motor end-plate became attenuated as they were transformed into the granules of Kühne (Figs. 44, 47, and 48). This transformation of axons into granules was well advanced in some places (Figs. 48 and 49). The periodic internal structure of the muscle was replaced by a diffuse arrangement of granules (Fig. 49).

These masses of discharged nervous secretion into the muscle underwent progressive decrease in size and incorporation into the colloidal substance of the myoplasm of the muscle fiber. It was clearly evident, therefore, that there was no fixed boundary or line of demarcation between the junction of nerve and muscle. The substantial secretion from the motor nerve ending became incorporated into the explosive mixture that forms the active muscle.

There were many examples of so-called ultraterminal branches (Figs. 18 to 22) of the motor end-plates in the very early periods after shock due to scalding. The term "ultraterminal" was applied to an unmyelinated branch which arises from the ramifications of the axonic terminals of the motor end-plates. These ultraterminal branches terminated in rudimentary swellings in the same or in neighboring muscle fibers. Both types of terminals have been observed. They were the product of overstimulation due to multiple causes. They were frequently found in the very early stages of primary shock due to scalding of the skin of rats. They represented an early stage in the explosive projection of axonic secretory material from the motor end-plates due to reflex overstimulation. They were found under various conditions of anoxia. At another instant of time the projected material became disconnected from its point of origin in the axonic branches of the motor end-plates. These projected secretory masses of axonic material then became discrete bodies of inclusions in the myoplasm of the muscle fiber. This stage of axonic inclusion masses in muscle was a very temporary one. The nervous material discharged into muscle soon underwent dissolution. The accumulation of acids and other metabo-

lites in muscle due to overstimulation and anoxia causes a delay in the the dissolution of acetylcholine deposited physiologically at the synapses, according to Gesell and co-workers.[13]

The end-plates showing these sequences of changes of their hypolemmal axons following the reflex activity of scalding the skin—(1) hyperchrysophilia, (2) axonorrhea, (3) hypochrysophilia, (4) achrysophilia, and (5) centripetal degeneration or depletion of the epilemmal axons—were intermingled with relatively normal end-plates. Large quantities of muscle tissue were teased in order to reveal the relative proportion of the pathologic changes of the end-plates. Certain axons of a motor tree manifested excessive pathologic changes; others remained relatively normal in the same or neighboring trees of innervation.

4. *The Centripetal Depletion of the Epilemmal Axons.* The gastrocnemius muscle (Fig. 74) was excised 2 hours after the entire body of the rat, except the head and neck, had been immersed for 10 seconds in water at 75° C. There was complete disappearance of the motor end-plates (achrysophilia) and diminution of the capacity of the granular epilemmal axons for gold impregnation (hypochrysophilia). The depletion of these axons extended in a centripetal direction and away from the site of the granules that occupied the previous site of the motor end-plates. Thermal shock, therefore, produced a structural exhaustion of both the epilemmal axons, that extended proximally for variable distances, and hypolemmal axons of the motor end-plates.

5. *The Pathologic Changes in the Muscle Fibers.* The time of appearance of spastic and flaccid paralysis depended upon individual resistance of the rats, upon the temperature of the water, and duration of immersion. The time interval after traumatic scalding was likewise a factor in the degree of intensity of pathologic changes in the muscle fibers. During the early period after scalding the cross striations in many places were replaced diffusely by granules (Figs. 49 and 74) arranged especially in close relationship to the motor end-plates. The muscles, whose irritability was reduced or lost, had Zenker's waxy degeneration localized in some fibers. There was increased visibility of pyknotic nuclei in certain muscle fibers. There was a loss of the differential types of fibers in the majority of the muscles examined microscopically. There was little alteration of the intramuscular capillaries during the very early periods, 10 to 60 seconds after scalding. These capillaries were dilated and packed with clumps of red blood cells in many places within the muscles 2 hours after the heat stimulus (75° C. for 10 seconds) was applied to the skin. During the early stages the lesions of the muscles were dominant in the neuromuscular apparatus.

During the late stage hyperemia, perivascular edema, and leukocytic infiltration were prominent findings.

By counterstaining with hemalum there were found clusters of dark, pyknotic, rounded nuclei, 6 to 15 (Figs. 68 to 70) in number, that were related to the sole plate. These nuclei appeared to be modified muscle nuclei. Within the cluster of nuclei of the depleted motor end-plates there was granulation, in some places, of the cross striations of the muscle fiber. These morphologic changes were comparable to those produced by the injection of lactic acid locally in the zone of innervation of the muscle. The epilemmal axons frequently ended in bulbous expansions (Figs. 68 to 70) and had complete depletion of their motor end-plates. The function of these sole plate nuclei is under investigation.

Discussion

The prevailing static concept of the relation of the motor end-plate to the striped muscle fiber is one of a mechanical penetration and crude apposition, of discreteness and separateness of two vitally united structures. This may be one effect of the cell theory based upon the concept that the muscle cell and nervous cell are completely discrete structures in all of their parts. The dynamic interrelationship of muscle and nerve is an acceptable point of view to account for the periodic discharge of nervous substances into muscle. This results in a blending of the neuromyoplasm in a functional dynamic unit.

The initial mixture of nerve and muscle working-substances quickly forms a compound of complex colloidal neuromyoplasm. The nervous discharge may be a fine spray or a flood of secreted substances. A complex explosive mixture of the nerve and muscle substances, like a mixture of mercury fulminate and black powder, becomes thoroughly commingled until the nerve substance is nondetectable as such in the neuromyoplasm. The periodic mild and strong chemical reactions produced by the explosive mixture in an elongated capillary strand of nerve and muscle substances, confined in one fiber, are reflected in either regular and ordered cross striations or irregular and completely disordered arrangements of the internal structure of the cross striations.

The cross striations of living, nerve-intact muscle, therefore, are the mechanical products and structural expressions of capillary chemism enclosed in an elongated capillary system of neuromyoplasm. During life there is a ceaseless replacement of one system of cross striations by another. There are variations of amplitude and of combinations of one system of periodic bands with another. There may be mathematical additions and subtractions of constructive and destructive interfer-

ences, respectively, of these various systems of longitudinal pressure waves, which are expressed in the variable internal structure of skeletal muscle. Any concept that attempts to simplify the internal structure of muscle, for descriptive purposes, by postulating constancy of sarcomeres and alphabetical symbols, does not conform to easily reproducible facts obtained by experimental morphology. This static concept of structure has hindered the correlation of the anatomy and physiology of nerve and muscle for over a century.

The cross striations are the structural expression of periodic pressure waves composed of alternate zones of condensation and rarefaction that accompany the periodic explosive chemical changes in the capillary skeletal muscle fiber. This fiber, therefore, is dual in nature: (1) Its chemical composition undergoes reversible and irreversible quantitative and qualitative changes that are periodic or aperiodic; and (2) the chemical reactions confined in capillary spaces are accompanied by the physical explosive energy of internal compression waves. These align the protoplasmic colloidal mixture of the skeletal neuromuscular fiber into longitudinal waves of alternate zones of compression and rarefaction. This spectrum of internal compression may be used to estimate both the intensity and speed of chemical reactions and of the physical internal pressure which is synchronous with the explosive chemical reaction. The revelation of the secret of the transformation of the chemical energy of muscle into mechanical work lies in the field of morphology correlated with that of chemistry, physiology, and pathology. The attached myoneural capillary fiber gives direction to the explosive action of the muscle protoplasm roughly analogous to the direction given by the gun barrel to the explosive action of its gun powder, but the gun barrel, the skeletal muscle fiber, is closed at both ends.

Thermal shock produces pyknotic changes of the nuclei of the granular sole of Kühne. The functions of these nuclei are unknown. Do they contribute to the synthesis of the hypothetical transmitter substance—acetylcholine? Do they aid in the manufacture of choline esterase? The combined methods of morphology and that used by Stoerk and Morpeth [14] in the estimation of choline esterase activity in skeletal muscle may aid in finding the answers to these questions.

The so-called bloody tears or chromodacryorrhea was considered by Tashiro and Stix [15] as the biologic effect of acetylcholine or dacryorrhetin. Dacryorrhetin was found in an extract of muscle and considered a product of muscle metabolism. There appeared to be a correlation between the abnormal changes in the harderian glands of the lacrymal

apparatus and those of the neuromuscular apparatus in thermal shock in the rats. Additional experimental work was suggested by this interrelationship.

From a very early period, the nature of the terminals of nerve in striped muscle has occupied the attention of anatomists and physiologists. Before the use of the microscope, conjecture alone was possible. It was supposed that the terminal twigs of a nerve broke up into finer and finer branches which became finally fused with the muscle. The nerve fiber was considered a conduit for the transmission of a vital spirit which in some manner was delivered to the organ activated by nerves.

That the nerve fuses and commingles with muscle by the discharge of some ephemeral and tenuous substance through the secretory mechanism of the motor end-plates is nearer the truth than anatomists have realized since the introduction of the microscope. The termination of nerve in muscle is one of a fusion, a commingling of their respective substances and not one of cellular separation by partitions, like watertight compartments. There is thus established a morphologic and functional continuity and dynamic unity of the organization of nerve and muscle.

The intact living muscle fiber with its blood supply and attachments should be looked upon as a morphologic and functional whole composed of neuromyoplasm. This applies to both red and white muscle fibers. This unit may have periodic changes in its internal structure of longitudinal and cross striations corresponding to changes of function and nutrition. The inconstant longitudinal striations are evanescent mechanical products of streamlining produced by metabolism and the mechanical tension and pressure due to changes of function and nutrition. The specious division of the muscle fiber into stable contractile myofibrils and constant, intermediate, nutritional sarcoplasm does not conform to the facts. Some muscle fibers have well defined longitudinal striations and granular sarcoplasm; other neighboring fibers in the same muscle do not possess longitudinal myofibrils. These morphologic differences are correlated with different levels of metabolism and functional activity. One type readily transforms into the other, but both stages perform their tonic, thermal, and contractile functions. The muscle fiber is activated as a whole throughout its neuromyoplasm. No adequate evidence has been presented to support the assumption that the ephemeral, longitudinal myofibrils of the muscle fiber are the only structures capable of contraction in the

muscle fiber. The neuromyoplasm of the skeletal muscle fiber, with or without a sarcolemma, functions as a whole; it may or may not be cross striated; it may or may not be longitudinally striated into ephemeral myofibrils.

The histologic characteristics of the dorsal deep component of the sternocleidomastoid muscle in the rat are those of a specific red muscle fiber. Its fibers are smaller in diameter on the average and more granular than the easily separated, ventral, superficial part of the muscle. The inclusion of pigmented granules of myohemoglobin evidently acts as a storage of oxygen for the intrinsic respiration and metabolism of the deep part of the muscle.

The red pigment of hemoglobin compounds is found in the larval blood-worms inhabiting the warm springs of the Yellowstone National Park. Brues [16] and Leitch [17] have studied experimentally the rôle of hemoglobin in the larval blood-worms of the dipteric insect, Chironomus. They came to the conclusion that the function of hemoglobin in the blood-worm and snail in thermal springs consists in making available, by the power of binding oxygen chemically, a quantity of oxygen sufficient for the needs of the animal at oxygen tensions so low that the necessary amount is not supplied by physical solution. There is a dearth of dissolved oxygen in thermal waters which renders respiration difficult for purely aquatic animals. Hemoglobin is characteristic of many mud-inhabiting invertebrates that live where oxygen is present in only very small amounts. The larvae of Chironomus, by the presence of hemoglobin, are thus able to live under relatively anaërobic conditions and, furthermore, an increased alkalinity serves in some way to increase the resistance of these animals, according to Packard,[18] to a lack of oxygen.

The presence of a muscle pigment of hemoglobin would give a local supply of oxygen within the muscle where there was a decrease of the availability of oxygen. Whether or not white muscle could be transformed into red muscle by elevations in temperature, much as Huggins and co-workers [19] have found that increase of temperature transforms yellow into red bone marrow, is a problem under investigation. Each red or white muscle fiber, respectively, functions as a unit and is composed of the chemical fusion of nerve and muscle substances to form the neuromyoplasm. The structural changes in both types of muscle fibers are correlated with different levels of functional activity. There are, however, physiologic differences between red and white muscle fibers that have not been completely correlated with structure.

Summary

1. Whole-mount specimens of the gastrocnemius muscle and the motor end-plates from 250 rats subjected to thermal shock produced by water immersion (except the head and neck) at 75° to 90° C. for 1 to 10 seconds, were studied by the gold and teasing method; the gastrocnemius muscles were excised from 20 rats 15 minutes after light nembutal anesthesia and used as controls. The muscles were excised from within 10 seconds to 3 hours after the thermal trauma. The muscles from the control series and the traumatic series were divided into groups and run through the gold technic together. Exact comparison of the quantity and quality of the gold impregnation in the relatively normal control series and in the traumatized series was made by this method.

2. There was individual variation in resistance of the rats to primary thermal shock. Some of the animals, immediately upon immersion, had severe clonic spasms and paroxysms, or convulsions that resulted in tonic extensor rigidity. Others manifested mild twitching at first, and then strong, shivering, fascicular movements of the muscles. After 1 to 3 hours many of the rats had flaccid paralysis of one or more extremities. There was variation in the transition from violent muscular movements and from a spastic to a flaccid state of the muscles, as well as in the degree and extent of loss of muscle motion. These functional changes were dependent upon: (1) individual resistance of the animals; (2) duration of immersion; (3) degree of temperature; and (4) time interval after onset of the violent heat trauma.

3. The temperature of the muscles varied from 37.8° to 42.4° C., immediately after immersion of 24 rats. There was very little direct conduction of heat, therefore, from the skin to the spastic skeletal muscle. It was concluded, therefore, that the initial and variable pathologic function of muscle in immediate response to cutaneous scalding was produced by violent impulses transmitted through the neuromuscular apparatus. Some of these muscles, during the early stage of heat shock, failed to respond to both direct and indirect electric stimulation. This likewise applied to certain flaccid muscles in the late stage of shock. There were variations of response of the muscles in both a generalized and localized manner.

4. The majority of the motor end-plates had disappeared in 75 per cent of the muscles examined within 1 to 3 hours. The ordinary sequence of histologic changes, intermingled with normal appearances, in the motor end-plates were as follows:

(a) Hyperchrysophilia or increased intensity of gold impregnation

due to increased quantity of axonic material transmitted to the motor end-plate.

(b) Axonorrhea or the abnormal discharge of the axonic material from the motor end-plates, increased in permeability, into the myoplasm of the muscle fiber. The substantial secretion from the motor end-plates was arranged in a unipolar, bipolar, and multipolar pattern. The pleomorphism of the discharged secretion was manifested as granules, vacuoles, short or long fusiform inclusion masses in the myoplasm, short or long Indian clubs, triangles, or irregular masses that had a greater affinity for gold than the myoplasm. The length of the axonic material secreted into muscle varied from 0.5 to 1000 μ. The nonmedullated projection of axonic material still connected with the motor end-plate has been previously described as an "ultraterminal branch." Such projections were frequently found during the early primary effects due to reflex overstimulation by scalding the skin. The finding of large amounts of inclusion masses of the discharged nervous secretion in the myoplasm was synchronous with the manifestation of rigid spasticity of the muscles. These nervous inclusion masses in the myoplasm were either opaque or cross-striated.

(c) Hypochrysophilia and achrysophilia were evident as a progressive lack of gold impregnation finally leading to the complete absence of gold in the terminal of the nerve. This lack of gold impregnation was due to the complete absence of the terminal axonic structures. These had undergone granular transformation and dispersion in the myoplasm. The final stage was a complete structural exhaustion of the motor end-plates coincident in time with the flaccid paralysis. There was muscle denervation at the myoneural junction produced by complete structural exhaustion. This exhaustion was the result of the sudden axonic transformation into granules which composed the products of the acute hypersecretion into muscle induced by scalding the skin. The abnormal heat stimulus to the skin accelerated the drainage and depletion of the axonic fluid in motor nerves.

(d) The granular exhaustion of the epilemmal axons extended in a centripetal direction. The motor end-plates appeared to be vulnerable chemical fuses between the peripheral nerves and muscles.

5. There were acute changes in the myoplasm in addition to the great quantities of inclusion masses of nervous secretion: granular, hyaline or Zenker's degeneration, and pyknosis of nuclei.

6. During the early stages, 10 to 60 seconds after traumatic scalding of the skin, the dominant change in the muscle was in the neuromuscular apparatus. After 1 hour there were prominent vascular

changes: active hyperemia, packing of the vascular lumina with red blood cells, perivascular edema, and leukocytic infiltration. There was individual variation of the rats in manifesting this usual sequence of pathologic reaction to the abnormal motor impulses generated by the reflex response to burns.

7. Ten per cent of the rats subjected to immersion in water, 75° C. for 10 seconds, manifested the so-called bloody tears or chromodacryorrhea of the harderian glands. It has been established previously that this biologic response was produced by acetylcholine or by the breakdown products of muscle metabolism called dacryorrhetin. There appeared to be a correlation in the isolated changes in the neuromuscular apparatus and in the harderian gland in the rat.

8. The nerve-intact skeletal muscle fiber should be considered as a functional and morphologic unit composed of neuromyoplasm; it may or may not possess a sarcolemma; it may or may not have cross striations; it may or may not be longitudinally striated or subdivided into myofibrils. The variable cross striations are structural expressions of the changeable mechanical energy of explosive pressure that accompanies the capillary chemistry of metabolism. The ephemeral longitudinal striations are streamlining products of the periodic changes of metabolism and of the mechanical tension and pressure accompanying muscle function. The cross and longitudinal striations are changeable and not constant structures. The myofibril, therefore, cannot be considered the constant specific structure that subserves the contractile function. It is a mechanical, structural effect and not the definite morphologic cause or sole protoplasmic substratum underlying muscle contraction.

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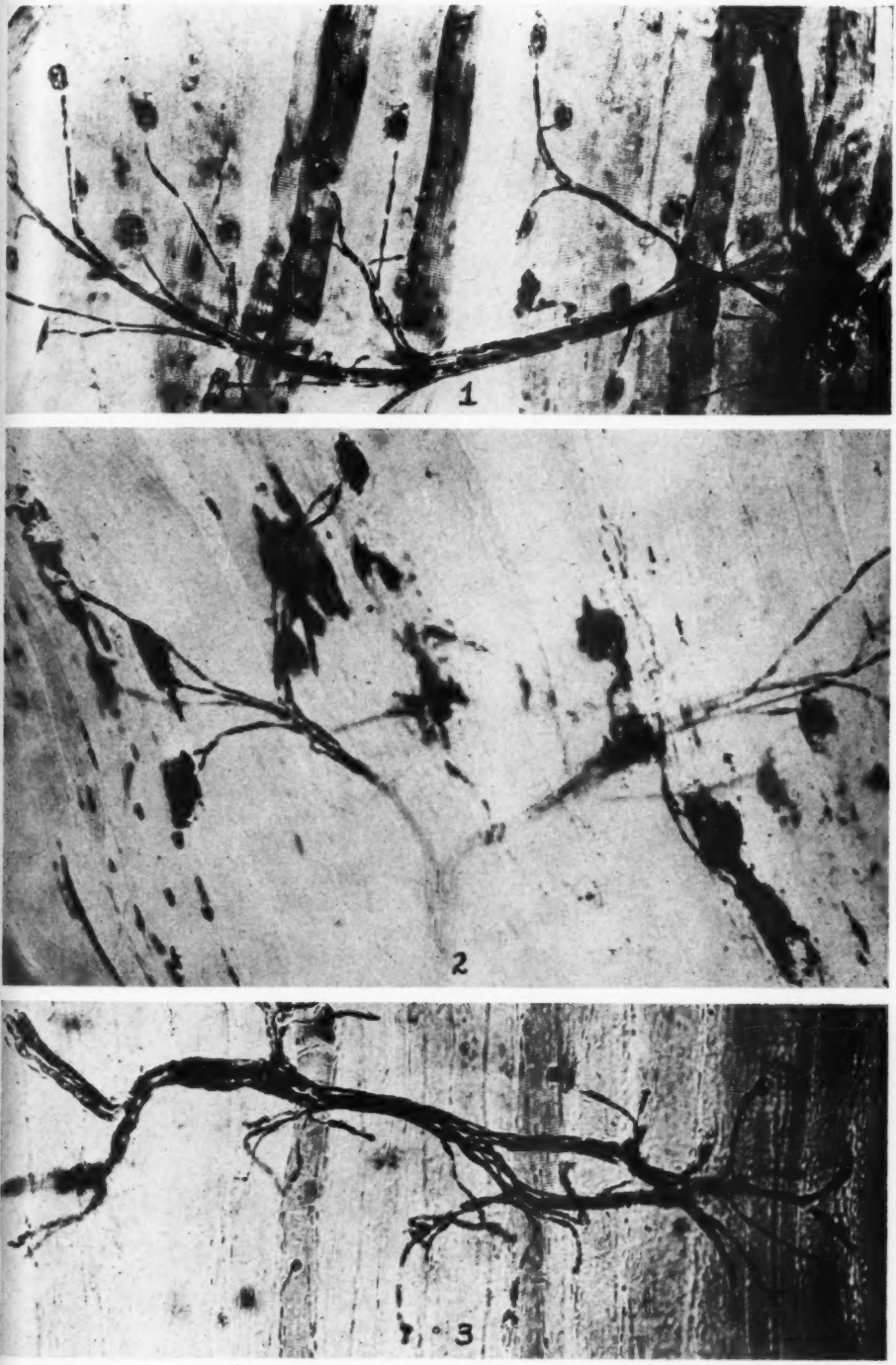
DESCRIPTION OF PLATES

The photomicrographs of Figures 1 to 71 are from teased whole muscle fibers (gastrocnemius muscle) and motor end-plates of the white rat (*Mus norvegicus*). These teased preparations of motor end-plates in skeletal muscle were previously prepared by the gold technic. The photographs were made as direct contact prints from the negatives which were exposed through the microscope and not subjected to subsequent enlargement. These photographs, therefore, may be readily compared with those of the the white rat and the chameleon previously published.[6] In the plates, "epa." designates the epilemmal axon; "hya.," the hypolemmal axon; and "Kg.," masses of extra-axonic Kühne's granules. There has been no retouching of either negatives or prints.

PLATE 36

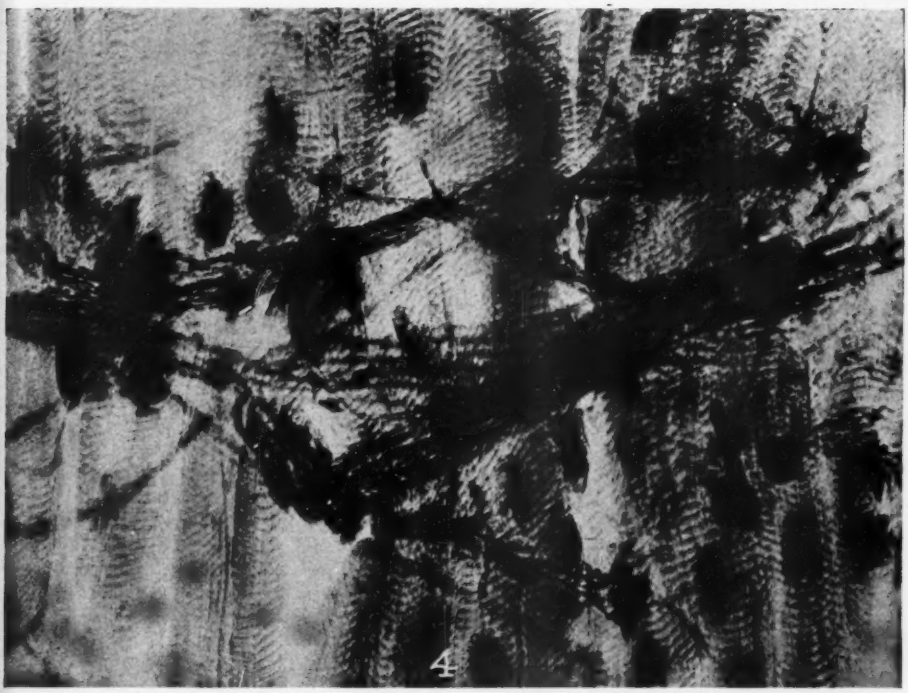
FIGS. 1 to 3. Sprays of axons of medullated nerve fibers and motor end-plates of the white rat (*Mus norvegicus*) in the relatively normal gastrocnemius muscle excised 15 minutes after the intraperitoneal injection of nembutal, 4 mg. per kg. (Fig. 1); muscle excised 30 seconds after immersion of rat at 90° C. for 10 seconds (Fig. 2); muscle excised 2 hours after immersion of rat at 75° C. for 10 seconds (Fig. 3). In the relatively normal muscle (Fig. 1) there is a variation in the type of muscle fibers and in the sizes of the motor end-plates. Some motor end-plates in the retracted condition are surrounded by a dense accumulation of Kühne's granules. The light, expanded end-plates have a diminution or complete absence of the surrounding granules of Kühne. The first morphologic effect of the heat stimulus applied to the skin was hyperchrysophilia (Fig. 2), increased intensity of gold impregnation of the enlarged motor end-plates. This was due to the increased accumulation of nervous material in the end-plate prior to discharge into the muscle. In some locations the discharged nervous secretion is evident in the muscle fiber. The epilemmal axons (Fig. 2) manifest beginning exhaustion of their substance. The complete dissolution of the motor end-plates (Fig. 3), and the centripetal depletion of the epilemmal axons of gold-impregnated substance are the last events in a whole series of changes that occur in the peripheral denervation of muscle at the myoneural junction subsequent to the shock stimulus produced by heat applied to the skin. The neuromuscular apparatus, therefore, manifests in thermal shock the following sequence of changes: (1) hyperchrysophilia of the enlarged end-plates; (2) axonorrhea, or abnormal discharge of nervous secretion into muscle from the abnormally permeable motor end-plates; (3) hypochrysophilia; (4) achrysophilia; and (5) depletion of the gold-impregnated material in the epilemmal axon in a centripetal direction. One clearly evident result of the reflex action of heat applied to the skin is the loss of the dark type of muscle fiber (Figs. 2 and 3) due to simultaneous contractions of the great majority of these fibers. The fractional contraction of normal muscle is structurally expressed by the presence of dark and light muscle fibers (Fig. 1). × 150.



Carey, Massopust, Zeit, and Haushalter Motor End-Plates in Thermal Shock

PLATE 37

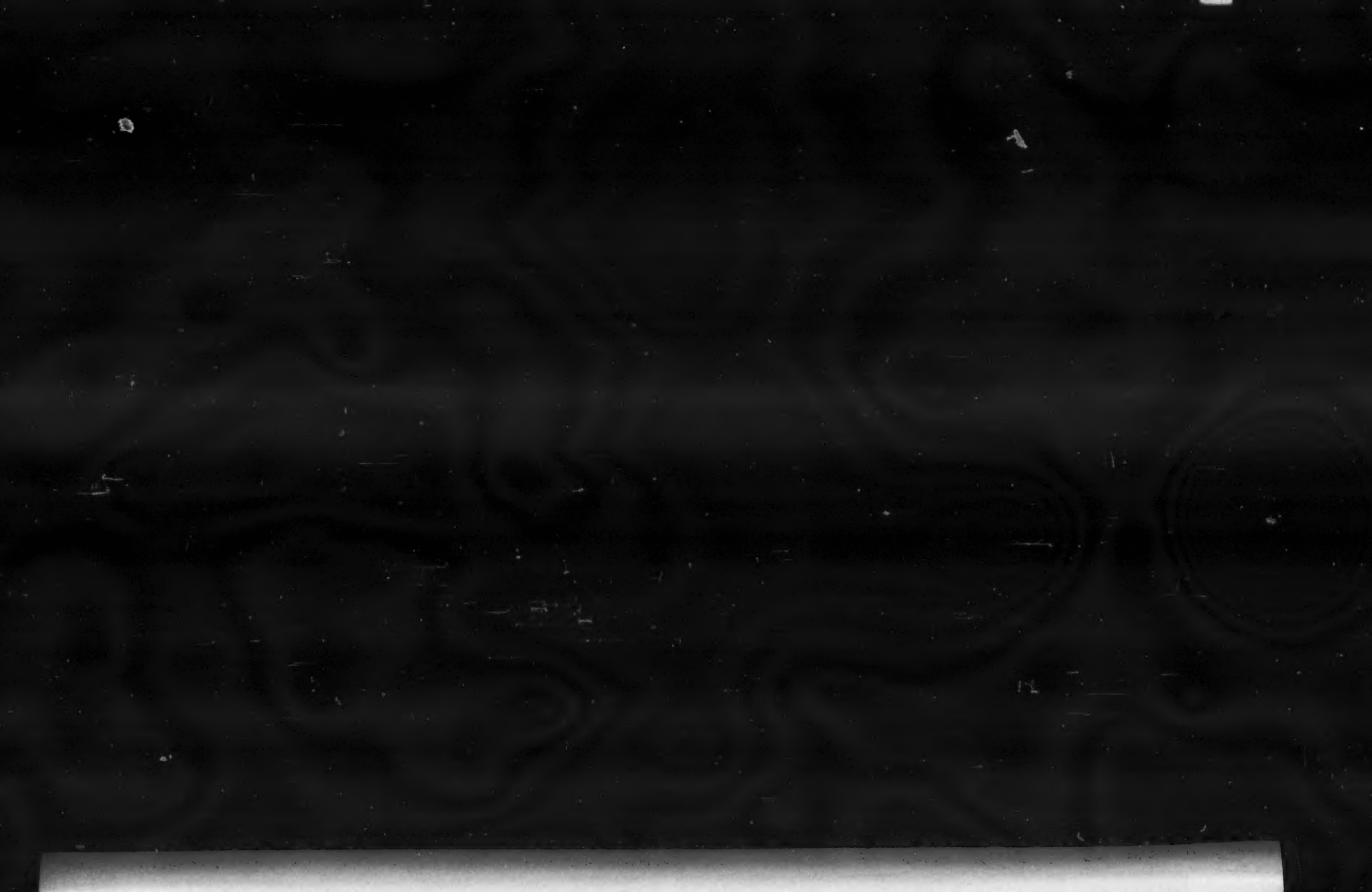
FIGS. 4 and 5. Sprays of axons of medullated nerve fibers and motor end-plates of the gastrocnemius muscle of the white rat, 4 hours subsequent to the intraperitoneal injection of magnesium sulfate, 2 gm. per kg. (Fig. 4), and 10 minutes subsequent to the subcutaneous injection of strychnine sulfate (Fig. 5). There is a great retraction of about 35 per cent of the motor end-plates in the gastrocnemius muscle after the injection of magnesium sulfate. The epilemmal axons are faintly impregnated with gold. The muscle fibers are narrow and the cross striations form coarse bands. The muscle is in a state of flaccid paralysis. Some muscles, in flaccid paralysis due to magnesium sulfate, failed to respond to indirect stimulation of the sciatic nerve. By direct stimulation the muscles responded feebly to electric stimulation. The above changes (Fig. 4) are to be contrasted with those produced by strychnine sulfate (Fig. 5). Ninety-five per cent of the endings are expanded, and in some places the hypolemmal axon is fragmented (Fig. 5) into discrete granules, which give the stippled appearance. The epilemmal axons are wide in some places and very narrow in others and are intensely impregnated with gold. About 95 per cent of the muscle fibers have fine, closely spaced cross striations. These changes of retraction and expansion of the motor end-plates and the configuration of the cross striations produced by chemical action are to be used as a base line of departure in the interpretation of the changes produced by thermal shock. × 300.

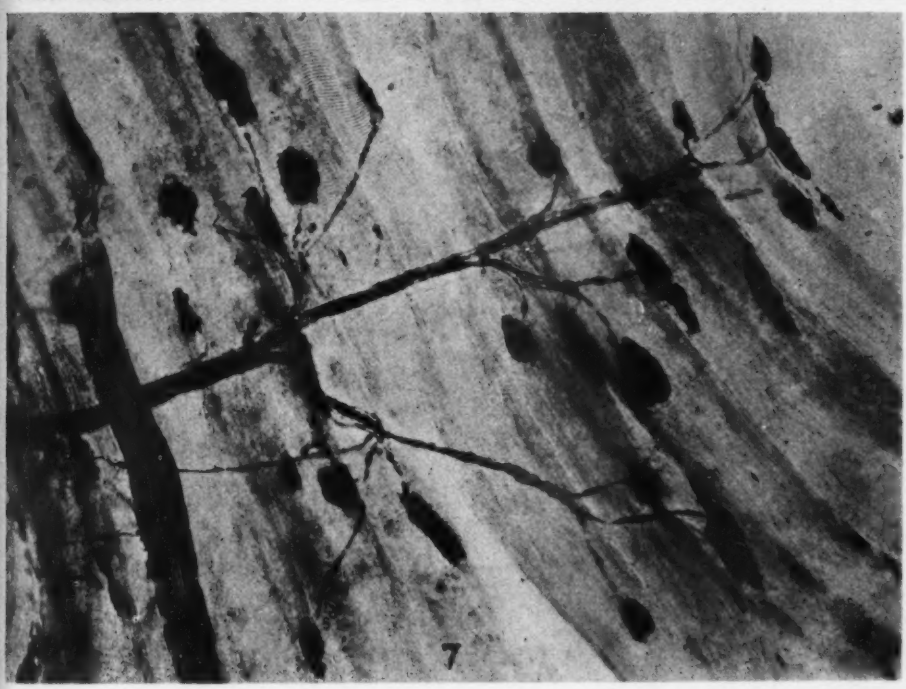


Carey, Massopust, Zeit, and Haushalter Motor End-Plates in Thermal Shock

PLATE 38

FIGS. 6 and 7. Sprays of axons of medullated nerve fibers and motor end-plates of the gastrocnemius muscle of the white rat, in the relatively normal animal (Fig. 6), obtained 15 minutes after the intraperitoneal injection of nembutal. There is variation in the type of muscle fibers and in the sizes of the motor end-plates. Some motor end-plates in the retracted condition are surrounded by a dense accumulation of Kühne's granules. The light, expanded end-plates have a diminution or complete absence of the surrounding granules of Kühne. It is evident that the first morphologic effect of the heat stimulus of a 10-second skin exposure in water at 90° C. is a hyperchrysophilia of the motor end-plates (Fig. 7). The muscle was excised 10 seconds after immersion. An augmented discharge of masses of Kühne's granules from the end-plates is made evident by the intense impregnation of this nervous material by gold. There is a beginning depletion and beading of the epilemmal axons. This phase of the secretion of the nervous material into the muscle fiber is a very early and ephemeral one. It soon gives way to explosive projection, diffusion, and subsequent dissolution of this nervous material in the myoplasm of the muscle fiber. × 150.

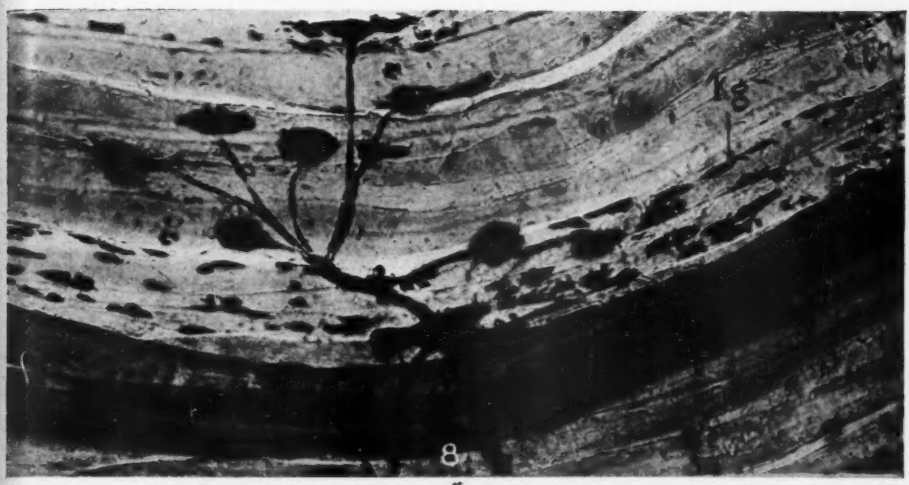




Carey, Massopust, Zeit, and Haushalter Motor End-Plates in Thermal Shock

PLATE 39

FIGS. 8 to 10. Sprays of axons of medullated nerve fibers and motor end-plates of the gastrocnemius muscle of the white rat, 30 seconds subsequent to the heat stimulus of a 10-second skin exposure in water at 90° C. The first phase of the neuromuscular reaction of hyperchrysophilia and the second phase of axonorrhea, or discharge of the nervous material into the muscle fibers, are intermingled. The discharged nervous material may be either close to or at a considerable distance from the terminal axons of the motor end-plates. The arrangement of the secretion in close proximity to the end-plates is unipolar, bipolar, and multipolar. The structure of the secreted material is likewise variable, namely: (1) as discrete granules, (2) as vacuoles, (3) as short or elongated fusiform masses, and (4) as short or long arrow-like projections. The internal structure of the masses of Kühne's granules projected from the motor end-plates into the muscle fiber may be either densely opaque or cross-striated. The cross striations, when present, may or may not agree in periodicity with the related cross striations in the muscle fiber. These projected axonic masses of Kühne's granules are seen both within and without the muscle fiber. This is more clearly revealed in the studies of the cross sections of the muscle fibers. There is a torrential projection of axonic material that, for a short period, floods the contents of some muscle fibers. This excessive discharge of the nervous secretion from the motor end-plates is detected by the superabundance of gold-staining masses that vary in size and configurations. The ephemeral architecture and multiplicity of designs of the discharged nervous secretion in muscle are consistent with the various steps occurring in the dissolution of the secretion and its progressive incorporation into the substance of the myoplasm. There is, therefore, no constant and fixed line of demarcation between the motor nerve ending and cross-striated muscle. This variation in composition of the junction between the secretory nerve ending and muscle has made the search for an absolute line of separation between the two structures vain; there is no such constant partition between nerve and muscle. × 300.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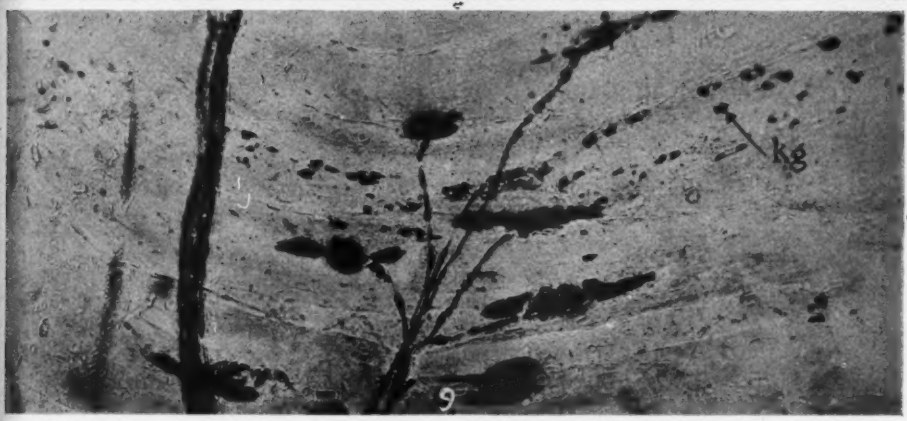
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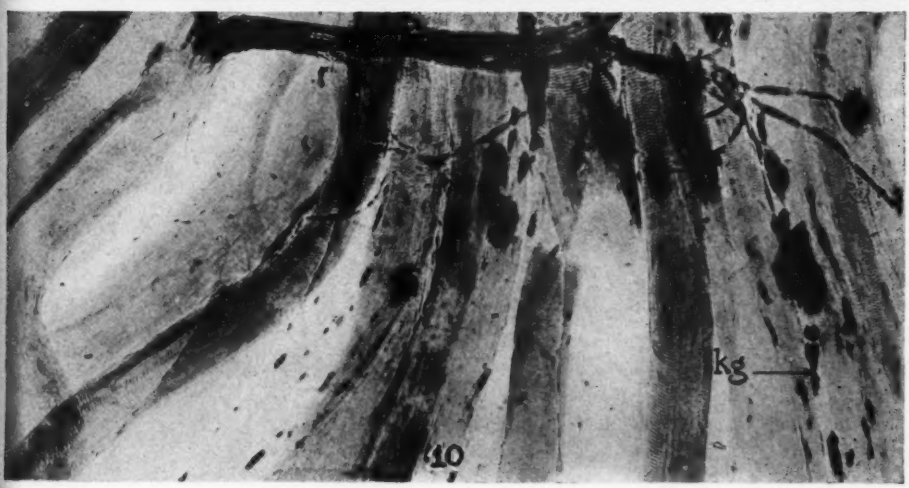
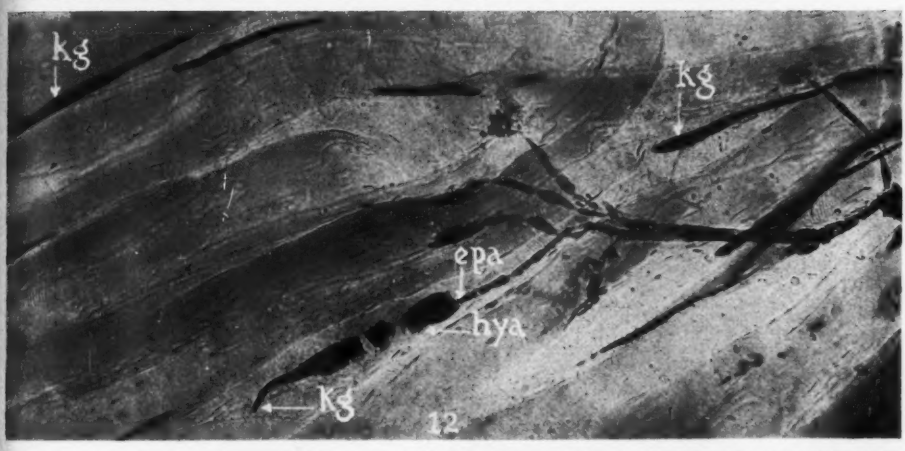

kg
10

PLATE 40

FIGS. 11 to 13. Sprays of axons of medullated nerve fibers and motor end-plates (Figs. 11 and 12) of the gastrocnemius muscle of the white rat, 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C. The second phase of the neuromuscular reaction, axonorrhea, is clearly manifested. Elongated streamers of gold-staining axonic material discharged from the motor end-plates into the muscle fibers are clearly evident. Some of these projected streamers of nervous material discharged into the muscle are over 1000 μ in length. At the other extreme of the variation in size, spheroidal granules, 0.5 to 1 μ in diameter, are to be found. There is, likewise, considerable variation in the distribution of this nervous secretion in the muscle fibers (Fig. 11). This is due to the irregularity of the nervous discharge as well as to the tenuous and ephemeral nature of the nervous secretion. The form of the nervous discharge (Kg.) is clearly evident: elongated fusiform masses, elongated arrowheads (shaped like an isosceles triangle, Fig. 12), or elongated Indian clubs (Fig. 13). There is, therefore, irregularity of the pattern of the discharged nervous material. The stimulus of heat applied to the skin likewise has a neoformative influence on the design of the motor end-plates in muscle. From some end-plates (Figs. 11 and 12) there is a vigorous outpouring of nervous secretion, impregnated black by gold, which forms elongated streamers in direct continuity with the end-plates. Figures 11 and 12, × 150; Figure 13, × 300.

kg
kg
11


kg
kg
epa
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12

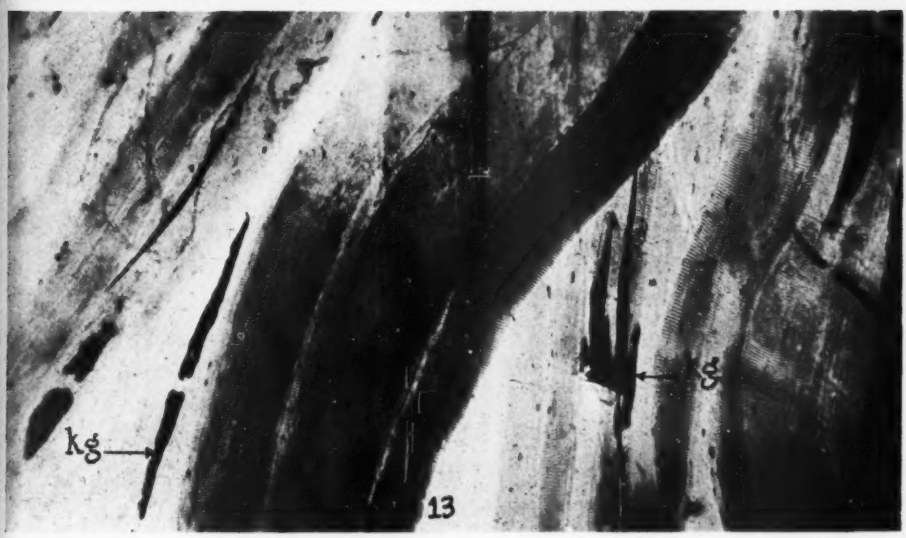
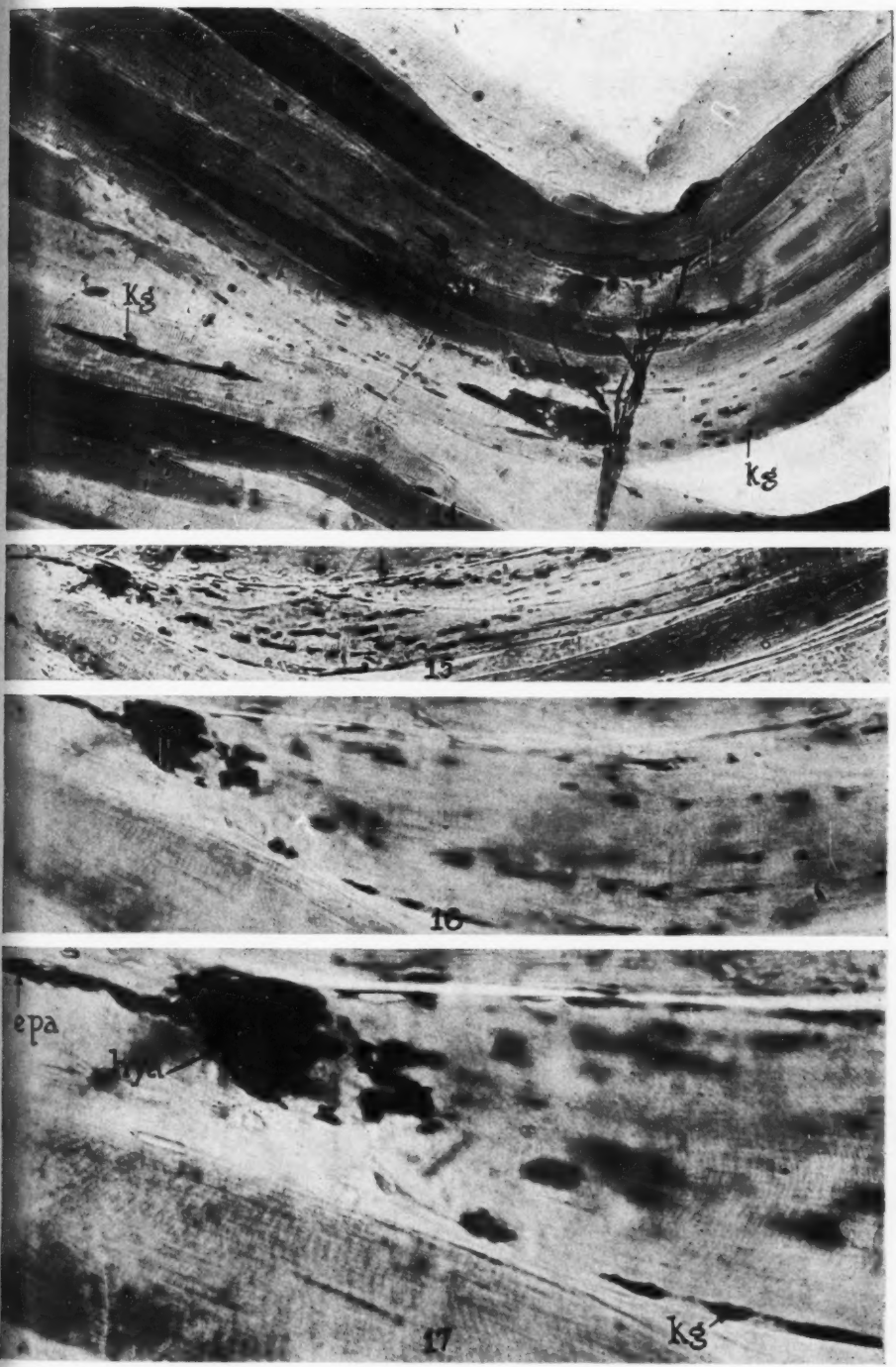

kg
g
13

Plate 41

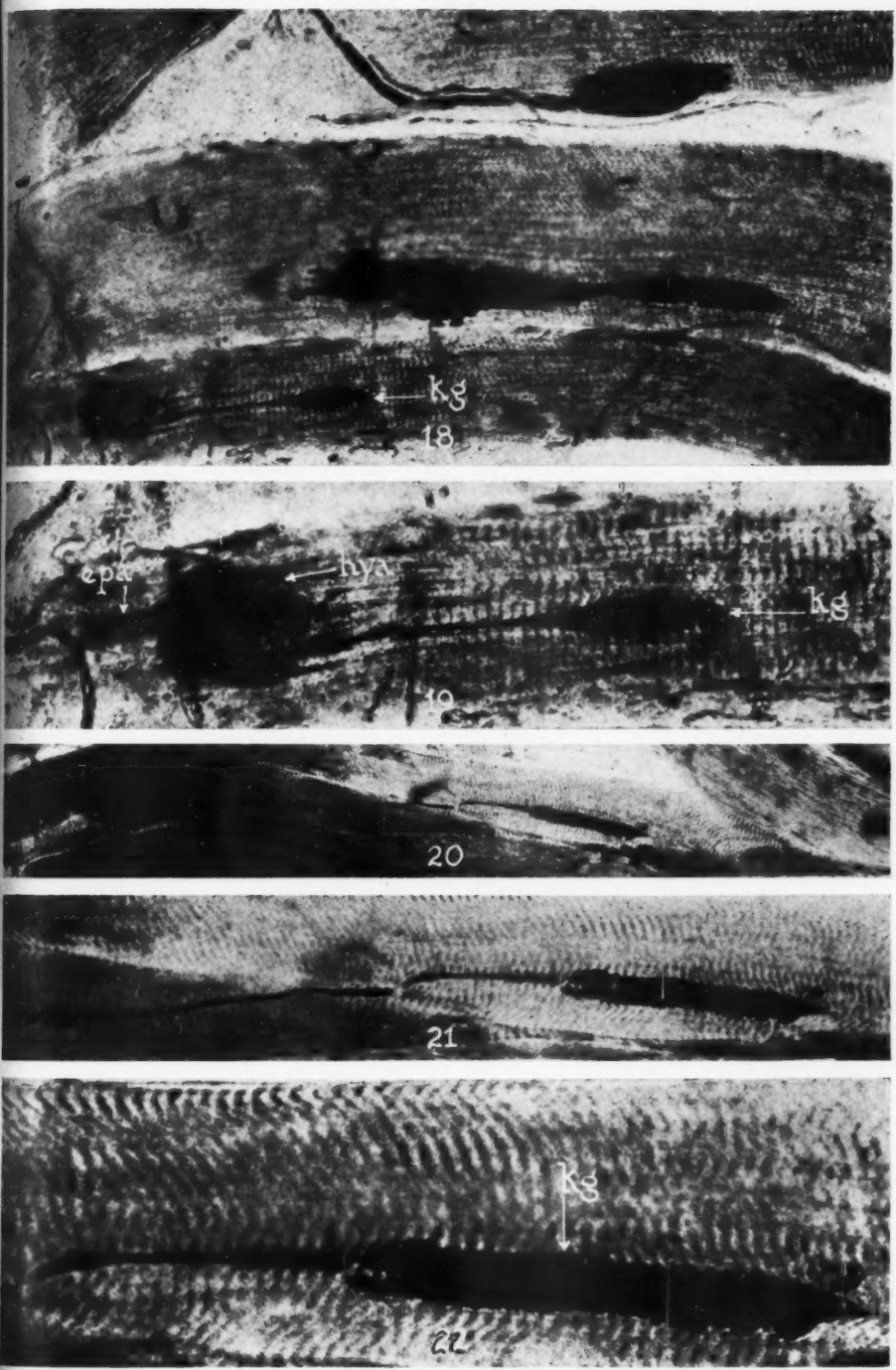
Figs. 14 to 17. Sprays of axons of medullated nerve fibers and motor end-plates (Fig. 14) of the gastrocnemius muscle of the white rat, 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C. The second phase of the neuromuscular reaction to heat applied to the skin, axonorrhea, is clearly manifested. The nervous secretion discharged from the motor end-plates is distributed both close to and at a considerable distance from the end-plates. Some of the secreted material is in the form of fine granules to the right of the lower motor end-plate (Kg., Fig. 14). The elongated streamer of nervous secretion to the left of the lower motor end-plate (Kg., Fig. 14) is 450 μ in length. In some places it is cross-striated and in others densely opaque. Some motor end-plates are fortuitously caught in the phase of pouring out a shower of nervous material into the muscle fiber. This is manifested (Figs. 15 to 17) in the various magnifications of the same motor end-plate and muscle fiber. Extending to the right of the discharging motor end-plate there is a flood of secreted nervous material, evident as dark, projected masses, into the myoplasm of the muscle fiber. There is morphologic evidence of a great flow of nervous material into the muscle by the streamlining of the very fine muscle striations. Figures 14 and 15, × 150; Figure 16, × 300; Figure 17, × 750.



Carey, Massopust, Zeit, and Haushalter Motor End-Plates in Thermal Shock

Plate 42

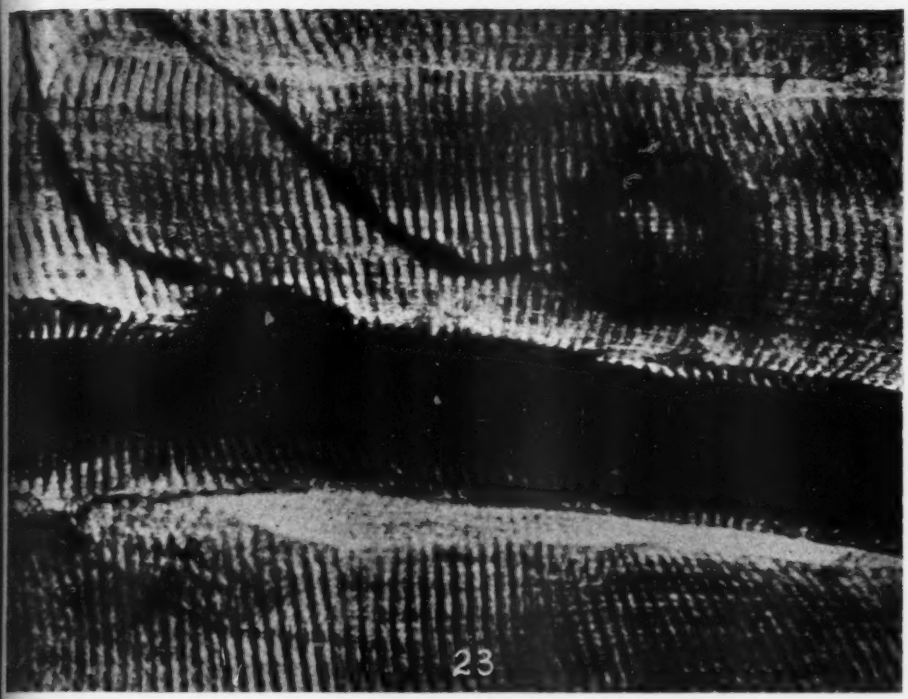
Figs. 18 to 22. Ultraterminal projections of axonic masses from the motor end-plates (Figs. 18 to 22) of the gastrocnemius muscle of the white rat, 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C. The second phase (axonorrhea) of the neuromuscular reaction to heat applied to the skin is manifested by many variations in structure. The term "ultraterminal" is applied to an unmyelinated projection which arises from the axonic material of the motor end-plate and which terminates in the same or in a neighboring fiber. Terminations of both types may be observed. The termination may be globular, fusiform (Figs. 18 and 19), or penniform (Figs. 20 to 22). There is an elongated axonic strand that connects the hypolemmal axons of the motor end-plate with the projected terminal mass. There is a definite streamlining of the labile cross striations of the muscle fiber in immediate relation to the terminal swellings (Figs. 20 to 22) of the ultraterminal projections of axonic material into the muscle fiber. These ultraterminal projections are intermediate phases in the discharge of the secretion of nervous material from the motor end-plates into the muscle fiber. At a slightly later stage the attenuated strand connecting the motor end-plate and the discharged mass undergoes solution. The continuity, therefore, of the discharged masses of Kühne's granules with the motor end-plate is an early stage in the manifestation of the secretion of nervous material into the cross-striated muscle fiber. This overflow of projected nervous substance into the muscle fiber is the result of overstimulation and augmented permeability of the terminal axons of motor nerves in muscle. The delay in the dissolution of the excessive nervous discharge may be due to the local increase of lactic acid and other metabolites in the overstimulated muscle. The continuation of this vigorous outpouring of the nervous fluid into muscle results in structural exhaustion and complete disappearance of the motor end-plates. This produces early denervation of muscle at the myoneural junction. Figure 18, × 300; Figure 19, × 750; Figure 20, × 200; Figure 21, × 400; Figure 22, × 750.



Carey, Massopust, Zeit, and Haushalter Motor End-Plates in Thermal Shock

PLATE 43

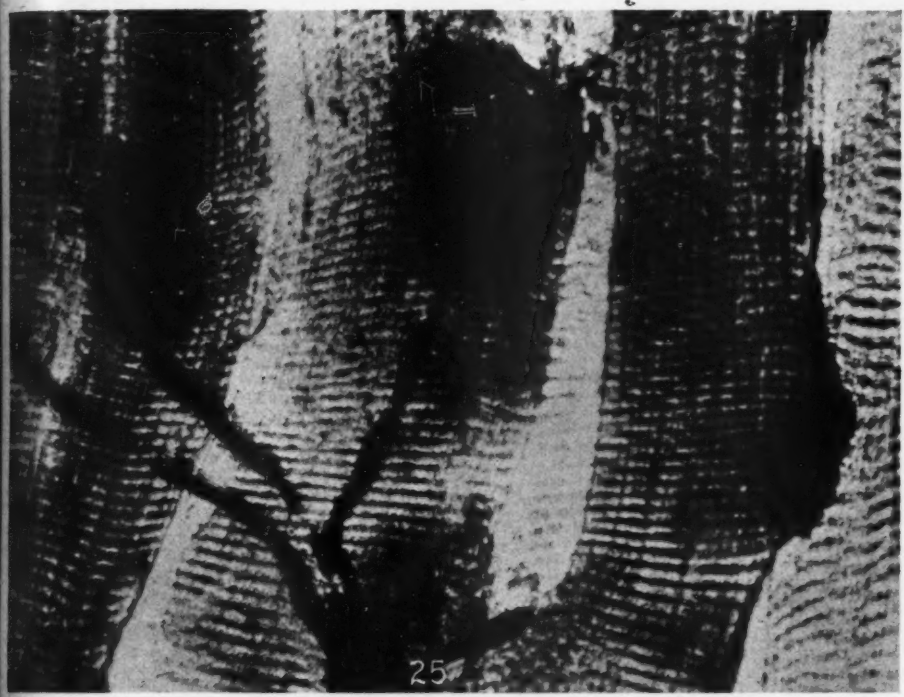
FIGS. 23 and 24. Sprays of axons of medullated nerve fibers in the relatively normal gastrocnemius muscle of the white rat, excised 15 minutes after the intraperitoneal injection of nembutal. There are variations in the width and types of muscle fibers in the relatively normal muscle. Some of the muscle fibers are narrow (Fig. 23) and densely impregnated with gold. Others are wide and less densely impregnated, giving a light appearance. There are many intermediate gradations in the width of the muscle fibers between the two extremes. The dark cross striations in the narrow muscle fibers are frequently broad, and the light banding narrow. The light, wide muscle fibers (Fig. 24) usually have fine, narrow cross striations. There are variations, however, in the internal structure of the muscle fiber depending on whether the muscle is fixed in the condition of isometric or isotonic contraction. The narrow muscle fibers may show a variation of fine striations, and the wide muscle fibers may show striations of the broad type. These labile cross striations, therefore, are not fixed, static membranes that form permanent partitions in the muscle. They are as variable as the functional activity and the chemical concentration and composition that underlie the activities of the muscle. The motor end-plates in the narrow muscle fibers are retracted and take a dense stain with gold. There are variations in the degree of expansion of the axonic terminals of the motor end-plates. There is a decrease in the quantity of the granules of Kühne around the expanded end-plates. There is a concomitant attenuation of the axonic branches in the expanded motor end-plates. Retracted motor end-plates have been associated with relaxation and expanded end-plates with contraction of the muscle fiber. The motor end-plates in this relatively normal control muscle are of the type designated as "*terminaison en plaque*." × 750.

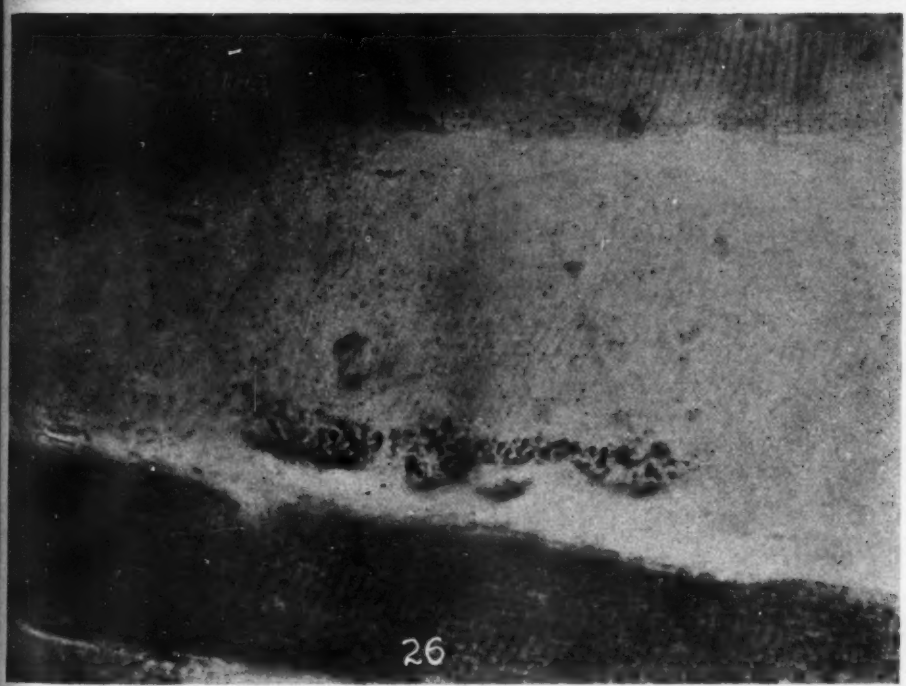


Carey, Massopust, Zeit, and Haushalter Motor End-Plates in Thermal Shock

PLATE 44

FIGS. 25 and 26. Sprays of axons of medullated nerve fibers in the relatively normal gastrocnemius muscle (Fig. 25) of the white rat, excised 15 minutes after the intraperitoneal injection of nembutal. The motor end-plates in this relatively normal muscle are of the type designated as *"terminaison en plaque."* Some of the end-plates (Fig. 26) in the gastrocnemius muscle, excised 10 seconds subsequent to the heat stimulus at 90° C. of a 10-second immersion of a rat, have the grape-like terminals designated as *"terminaison en grappe."* These grape-like terminals are an exhaustion phase of the ordinary motor end-plates due to overactivity and oversecretion in response to the heat stimulus. They are frequently associated with a muscle fiber that has fine, closely spaced, dark cross striations and with an unmedullated epilemmal axon. The demyelination of the epilemmal axon is, likewise, associated with exhaustion due to overstimulation. The reversible structure of the motor end-plates is due to variations of functional activity. The grape-like motor nerve ending has attenuated and, in many places, discrete swellings of the fine axons, which terminate in little ball-like masses. There is a depletion or complete absence of the surrounding granules of Kühne. The unmyelinated epilemmal axon of this grape-like nerve ending is out of focus when the ending is clearly in focus. × 750.

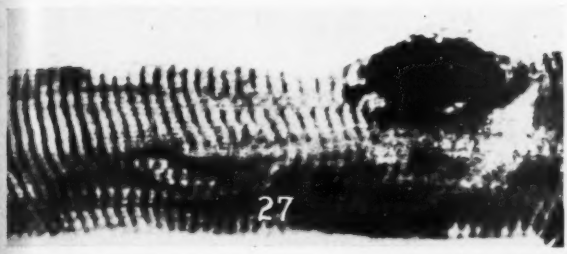




Carey, Massopust, Zeit, and Haushalter Motor End-Plates in Thermal Shock

PLATE 45

FIGS. 27 to 38. The pleomorphism of the normal motor end-plates is evident from these photographs made from the gastrocnemius muscle of the white rat in the relatively normal state, excised 15 minutes after the intraperitoneal injection of nembutal. Many of the retracted nerve endings (Figs. 27 and 28) are surrounded by a dense rim of Kühne's granules and are related to narrow muscle fibers that contain coarse, widely spaced cross striations. There is a great variety of configurations of the expanded motor end-plates. Some have coarse hypolemmal axons (Figs. 29, 30, 33 and 36) related either to fine cross striations or to a complete replacement of the striations by diffuse granulation. Other expanded nerve endings have very narrow hypolemmal axons and a great diminution or complete depletion of the granules of Kühne. The granules of Kühne constitute a fine spray of secretion discharged from the axons in the expanded nerve endings in some locations (Figs. 29, 31, 32, 36). These minute granules of Kühne become quickly incorporated by diffusion and dissolution into the myoplasm. Under normal conditions the neuronic secretion into muscle is exceedingly tenuous and ephemeral. Therefore, it has been hard to detect and interpret some of the clear, oval spaces between the branches of the axons. In the external granular rim they are occupied by nuclei of the granular sole. These nuclei are rendered more clearly visible by counterstaining with hemalum or hematoxylin. This counterstaining, however, frequently smudges the definition of the other parts of the neuromuscular apparatus. In some places there is a clear, halo-like space between the axon and the granules. In others there is a direct continuity between the granules and the axons. It is clearly evident that the architecture of the neuromuscular apparatus under relatively normal conditions is characterized by great variation and irregular patterns. The pleomorphism of these motor end-plates is due to variations of ameboid motion of retraction and expansion, and material exhaustion due to secretion. The epilemmal axon is irregularly beaded. × 750.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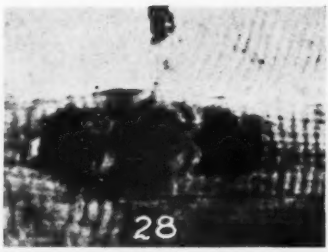
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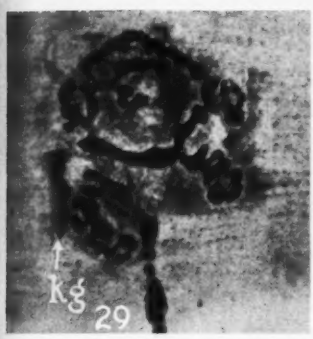
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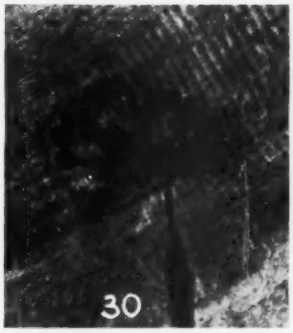
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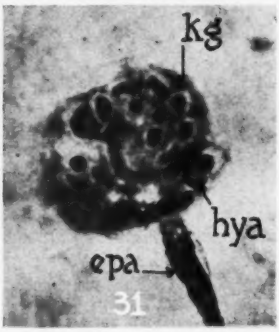
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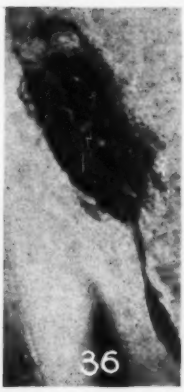
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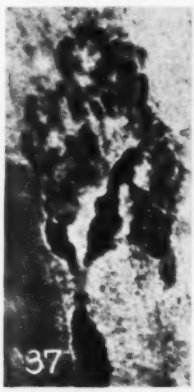
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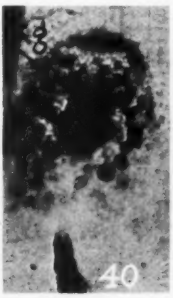
38

PLATE 46

FIGS. 39 to 49. The pleomorphism of the motor end-plates is clearly evident, also, in the gastrocnemius muscle of the white rat, excised 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C., applied to the skin. The hypolemmal axons may form a complete ring open in the center (Fig. 39), or may contain dense, rounded masses of Kühne's granules that form either a small (Fig. 40) or a large hub (Fig. 41). This central mass of Kühne's granules may form a ring with a thick rim (Fig. 42). There may be irregular distribution of the rounded islands of Kühne's granules (Fig. 43). There may be large rings (Fig. 44) or irregular rings (Fig. 45) formed by the expanded axons of the motor end-plates. The hypolemmal axons of the motor end-plates become gradually more and more attenuated as they become transformed into the granules of Kühne (Figs. 44 to 48). There is fragmentation (Fig. 47) and complete transformation of the hypolemmal axons of the motor end-plates into granules (Figs. 48 and 49). In this explosive transformation (Fig. 49), there is, likewise, a replacement of the periodic cross striations into an irregular mass of granules in close proximity to the end-plate which is undergoing rapid liquefaction. The epilemmal axons have irregular configurations in relation to the descent of the axonic material into the hypolemmal axons. The structural loss of innervation at the myoneural junction is due to the transformation of the terminal axons into granules. The rapid diffusion and incorporation of the granules into the myoplasm results in peripheral nervous exhaustion by depletion of the nervous axonic fluid discharged into muscle. × 750.

hya
epa
39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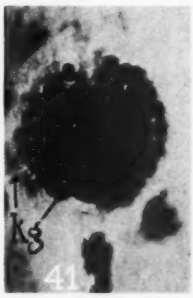
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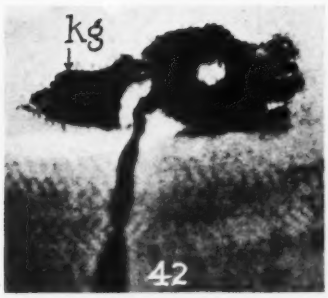
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kg
hya
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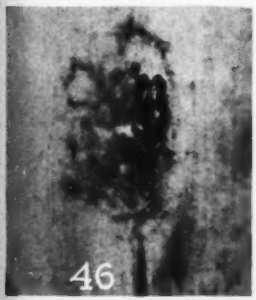
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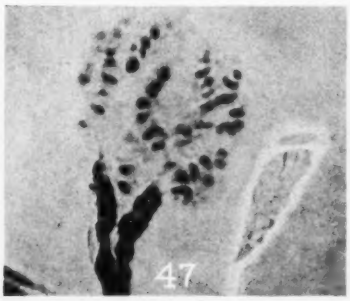
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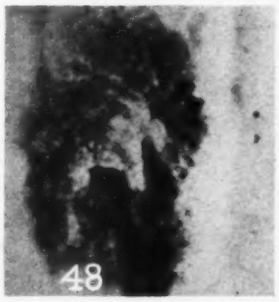
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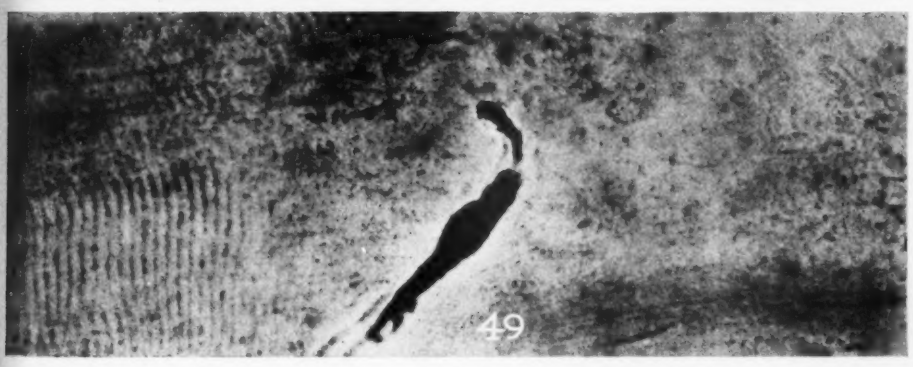
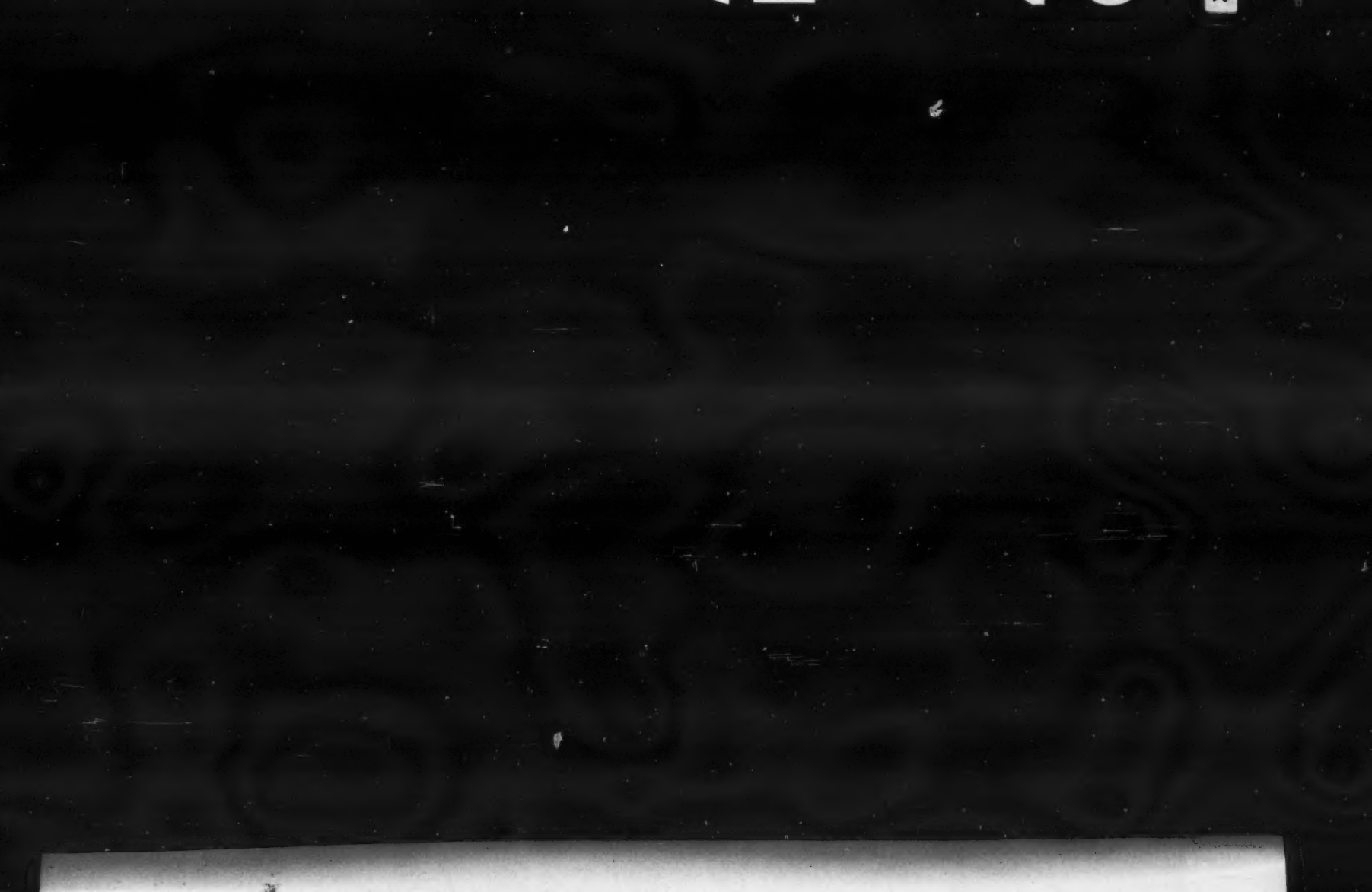
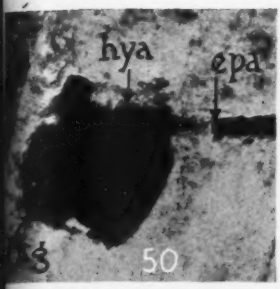
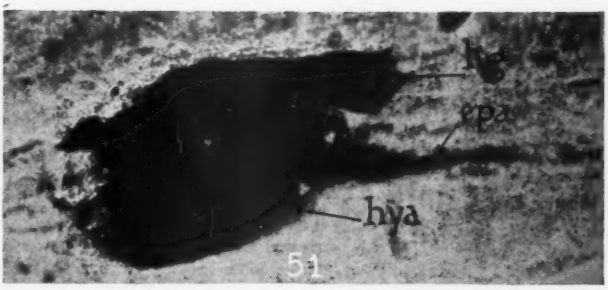

49

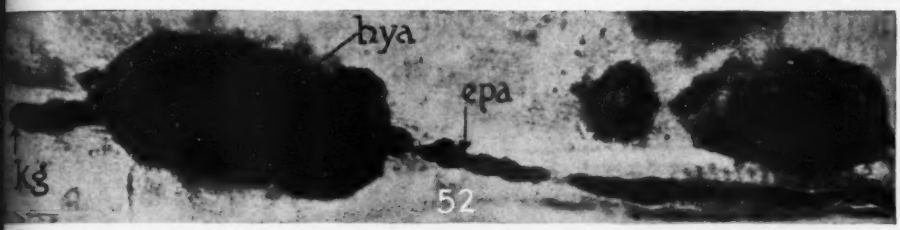
PLATE 47

FIGS. 50 to 55. The pleomorphism of the motor end-plates is illustrated further from the gastrocnemius muscle of the white rat, excised 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C., applied to the skin. The hypolemmal axon is in a condition of hyperchrysophilia due to the augmented accumulation of axonic material that has a specific affinity for the impregnating gold. This condition is not due to overstaining. The intense impregnation with gold is due to the fact that there is an increased quantitative accumulation of the axonic material in the end-plate, which material has an affinity for gold. There are various phases in the discharge of this nervous material into the muscle fiber by direct continuity from the motor end-plate. The elongated extensions of Kühne's granules may occur as short projections (Figs. 50, 51, 52), or as unipolar fusiform projections (Figs. 53 and 54), or as a duplex series of fusiform projections (Fig. 55), related to a depleted motor end-plate (Fig. 55). The fine cross striations are granular and closely spaced (Figs. 53 and 54) or they have undergone a complete replacement by a diffuse arrangement of granules (Figs. 50, 51, 52, and 55). × 750.

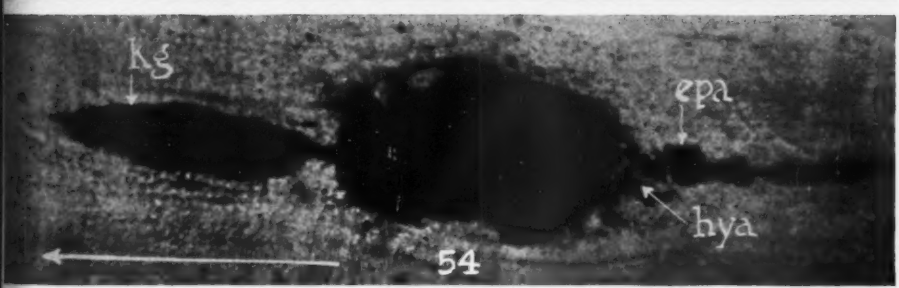



hya
epa
kg
50


kg
epa
hya
51


hya
epa
kg
52

kg
hya
epa
53

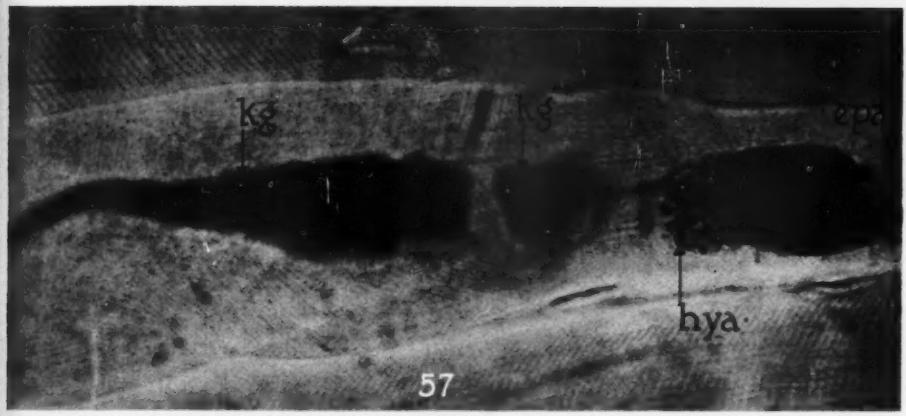

kg
epa
hya
54

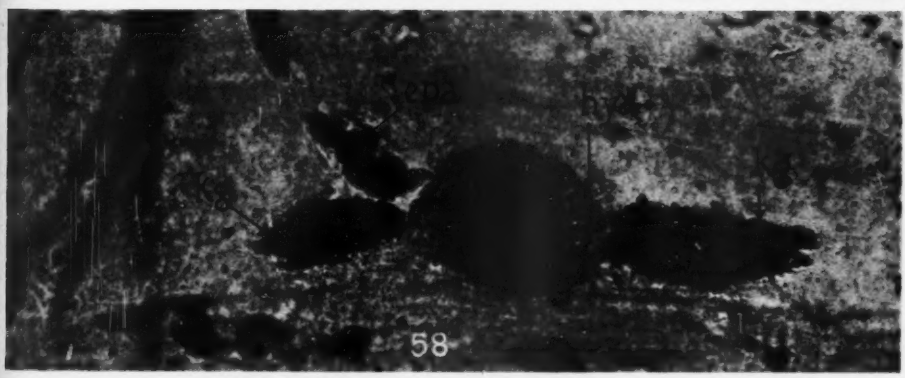
kg
kg
hya
epa
55

PLATE 48

FIGS. 56 to 59. The pleomorphism of the motor end-plates is illustrated from the gastrocnemius muscle of the white rat, excised 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C., applied to the skin. The hypolemmal axon is in a condition of hyperchrysophilia due to the augmented accumulation of axonic material that has a specific affinity for the impregnating gold. This condition is not due to overstaining. There is a unipolar disposition (Figs. 56 and 57) of the discharged masses of Kühne's granules to the left of the motor end-plates. There is a bipolar disposition of the discharged granules of Kühne (Figs. 58 and 59) to the left and to the right of the motor end-plates. The motor end-plate forms an open ring (Fig. 56) in some places, and in others the axonic ring of the end-plate encloses a dense island of discharged granules of Kühne (Figs. 57 to 59). In some places the cross striations are exceedingly fine and closely spaced (Fig. 57). In such places there is a streamlining effect produced on the pattern of the cross striations. This is evidence that these striations are easily displaced by the material from the nerve ending that becomes incorporated into the myoplasm of the muscle fiber. In other locations (Figs. 56, 58, and 59), the fine cross striations are undergoing replacement by a diffuse arrangement of granules. The alteration of the irregular configurations of both the motor end-plate and the cross-striated muscle occurs instantaneously and with explosive violence. By overstimulation of the neuromuscular apparatus, reflexly produced by heat applied to the skin, a flood of liquid nervous material with a variety of patterns is discharged into the muscle. × 750.

kg
h
epa
56


kg
kg
epa
hya
57


epa
58

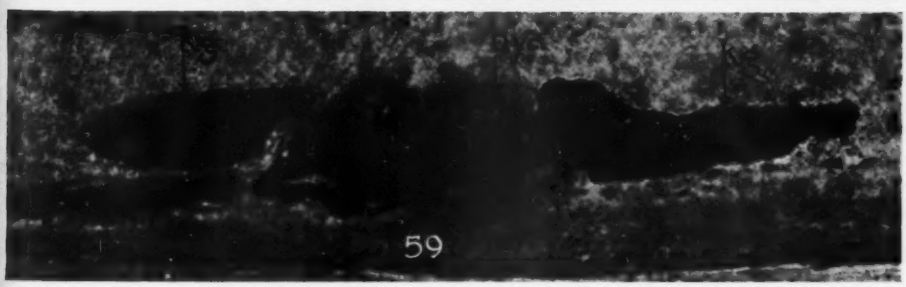
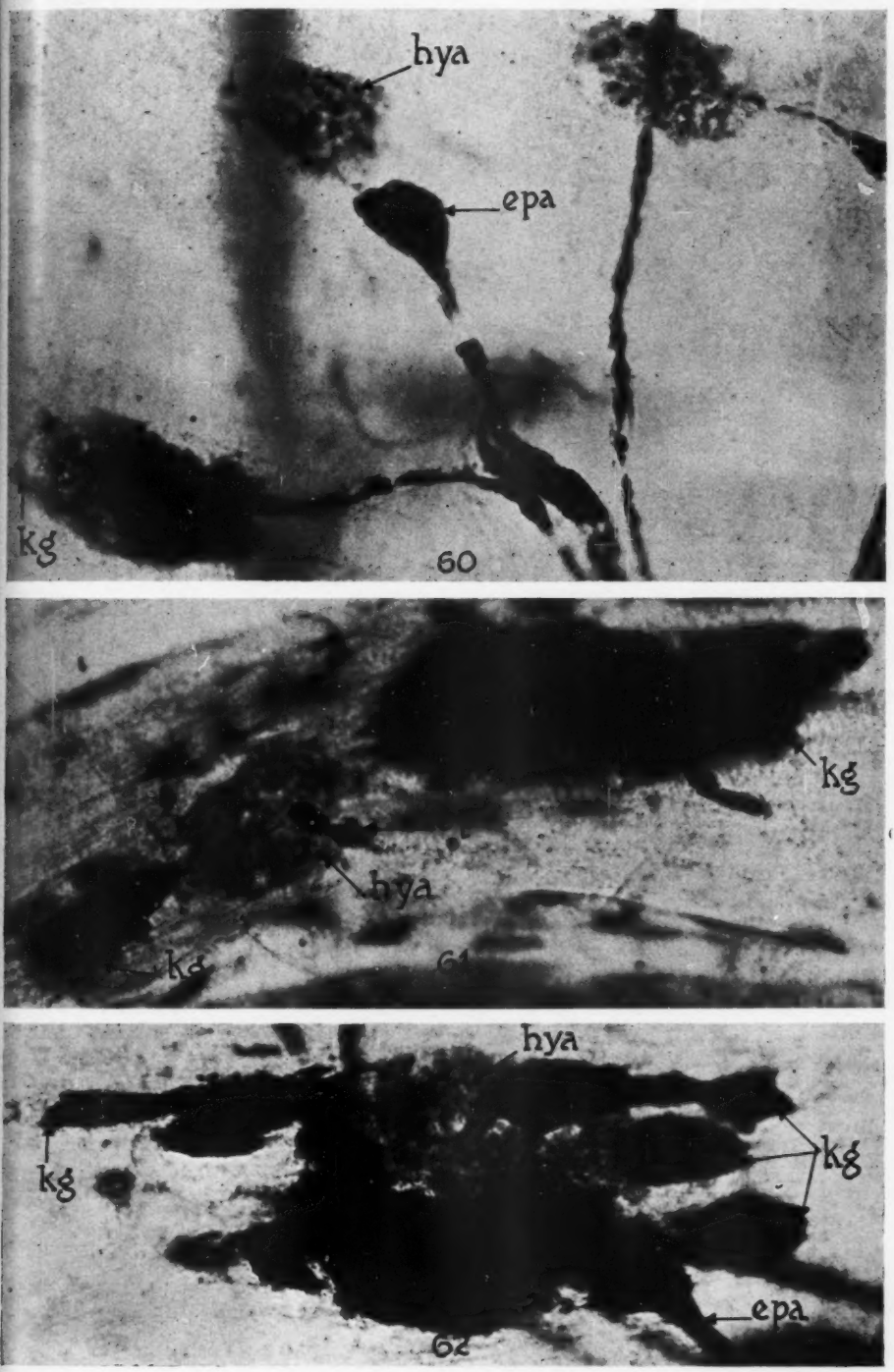

59

PLATE 49

FIGS. 60 to 62. The pleomorphism of the motor end-plates is clearly evident in the gastrocnemius muscle of the white rat, excised 30 seconds subsequent to the heat stimulus of a 10-second exposure in water at 90° C., applied to the skin. There is great variation in the width of the epilemmal axons (Fig. 60) in relation to the motor end-plates. This variation is consistent with the periodic descent of axonic material into the motor end-plates. The discharge of great masses of this material from the nerve ending into the muscle may be multipolar in arrangement (Figs. 61 and 62). The hypolemmal axons of the motor end-plates decrease in size in direct proportion to the transformation and discharge of the granules of Kühne. It is clearly evident that the heat stimulus applied to the skin has an immediate neoformative influence on the structure of the motor end-plate. The variations of design of the neuromuscular apparatus are related directly to variations of physiologic and pathologic activities. The relative disposition of the parts of the motor end-plates is not static and fixed, but is dynamic and highly variable in its architecture. × 750.

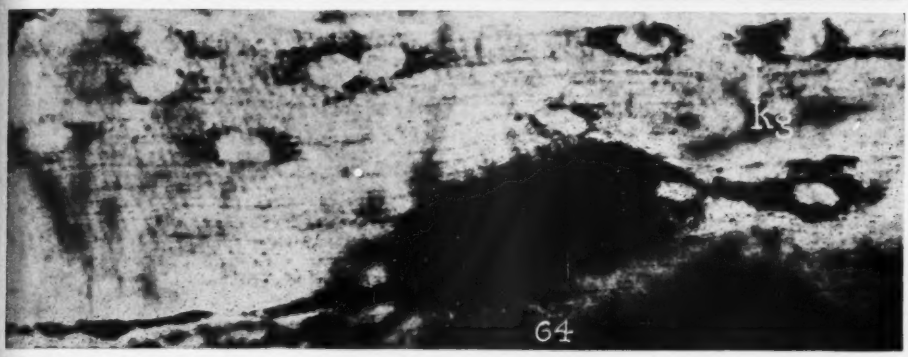


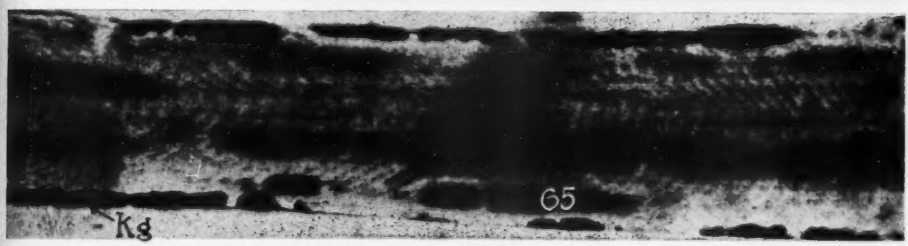
Carey, Massopust, Zeit, and Haushalter Motor End-Plates in Thermal Shock

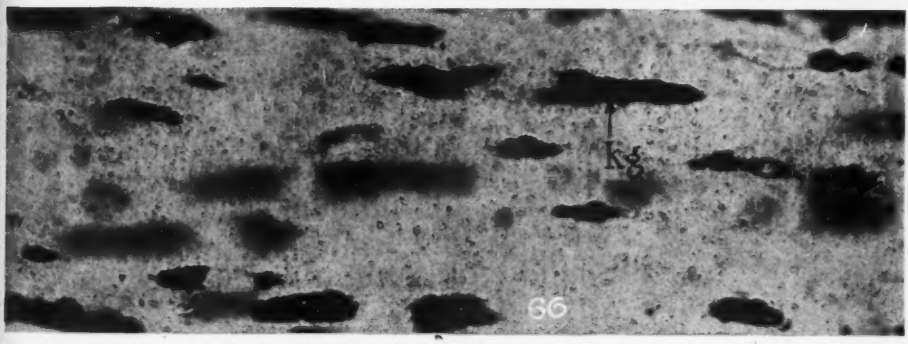
Plate 50

Figs. 63 to 67. The pleomorphism of the discharged granules of Kühne into the muscle fiber is clearly evident. These discharged granules are arranged in a variety of patterns, namely: discrete granules arranged in clumps or in series (Fig. 63); discrete or duplex vacuoles (Fig. 64); and elongated streamers or irregular masses (Figs. 65 to 67). It is clear that some of these masses lie within the muscle fiber and underneath the sarcolemma (Fig. 65). In other places they are found between the muscle fibers. In many locations the discharged secretion of the motor end-plates is dense and opaquely impregnated with gold. In other places these masses of Kühne's granules are cross-striated and their pattern of striation may or may not agree with that of the muscle fiber. These masses of nervous secretion discharged into the muscle undergo progressive dissolution and incorporation into the colloidal substance of the myoplasm of the muscle fiber. It is clearly evident, therefore, that there is no fixed boundary or line of demarcation at the junction of nerve and muscle. The substantial secretion from the motor nerve-ending becomes incorporated into the explosive mixture that forms muscle. The neuronic secretion into muscle may be compared roughly to the mercury fulminate that causes the explosion of black powder. × 750.

Kg
63


Kg
64


65
Kg


Kg
66

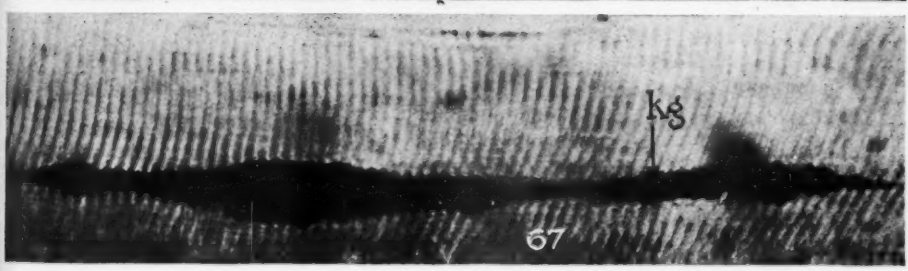
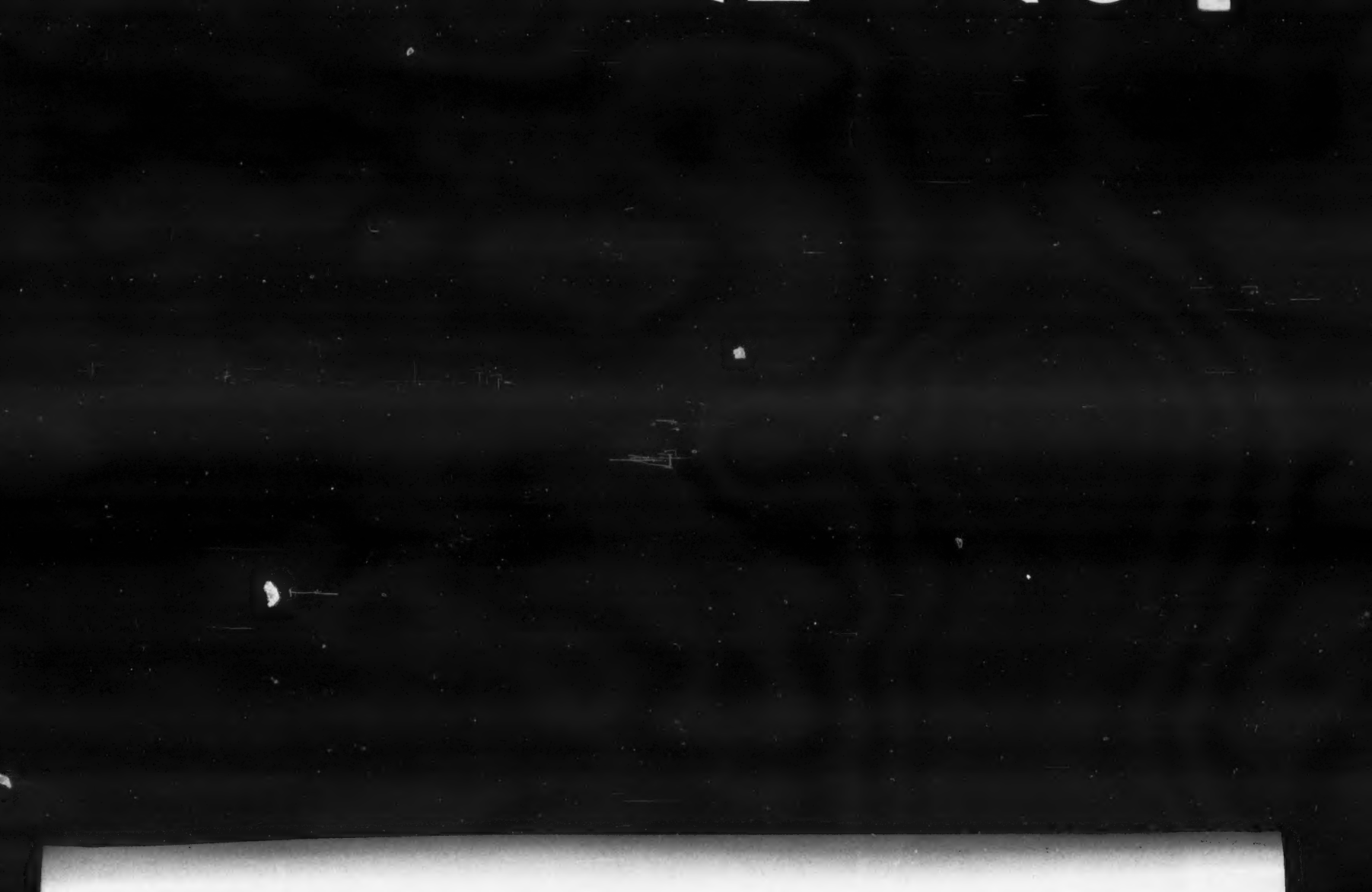
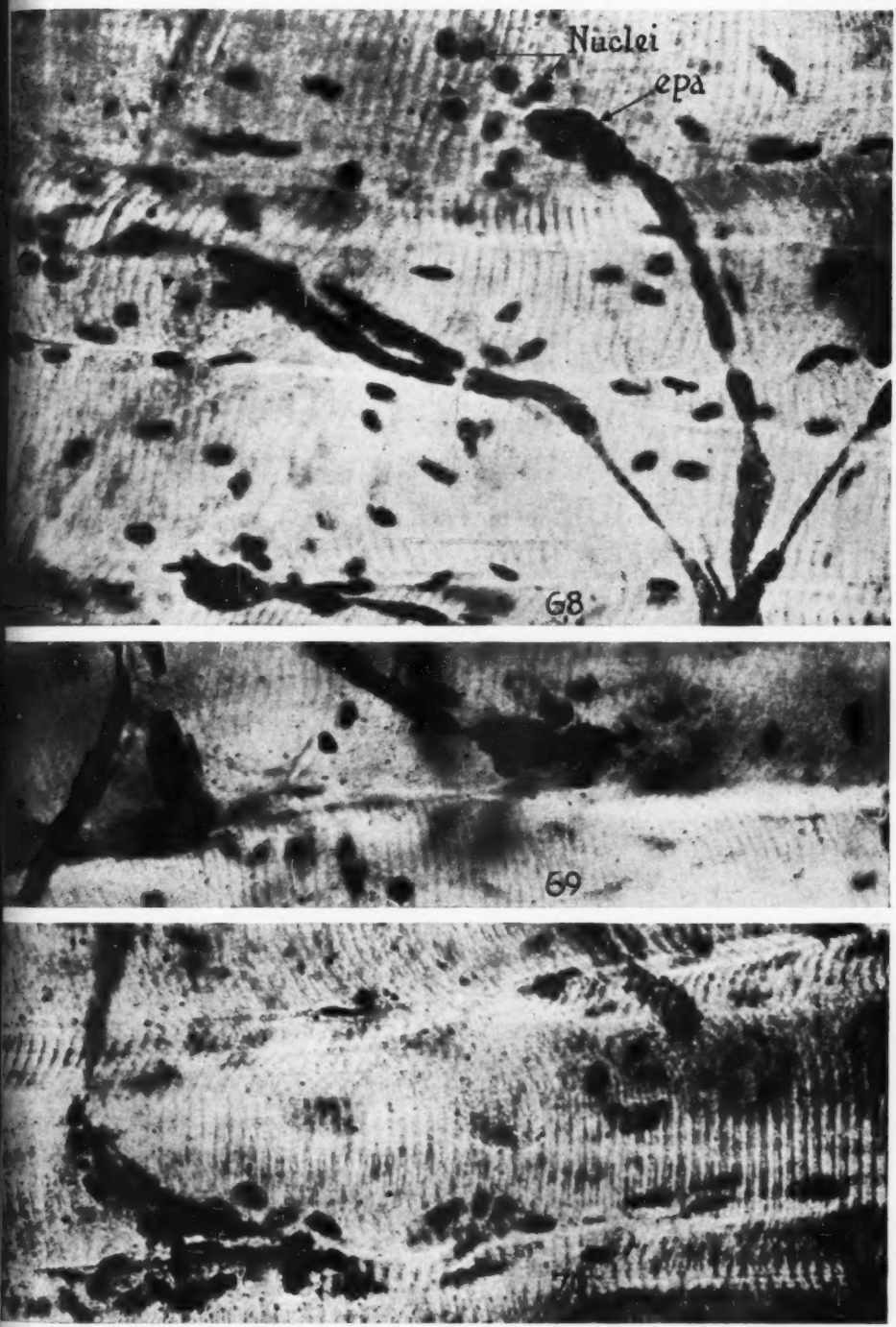

kg
67

PLATE 51

FIGS. 68 to 70. Sprays of epilemmal axons depleted of motor end-plates, 3 hours after scalding the skin of the rat in water at 75° C. for 5 seconds. There is both a hypochrysophilia and an achrysophilia corresponding to the progressive dissolution of the motor end-plates. The terminals of the epilemmal axons form enlarged ends. The hypolemmal axons have disappeared. In the previous location of the epilemmal axons are clusters of dark, pyknotic, rounded nuclei, 6 to 15 in number. These nuclei of the original granular sole of Kühne appear to be modified nuclei of the muscle fiber. Within the cluster of nuclei of the depleted motor end-plates there is granulation of some of the cross striations of the muscle fiber. These morphologic changes are comparable to those produced by the injection of lactic acid locally into the zone of innervation of the muscle. Gold chloride and hemalum stain. × 750.

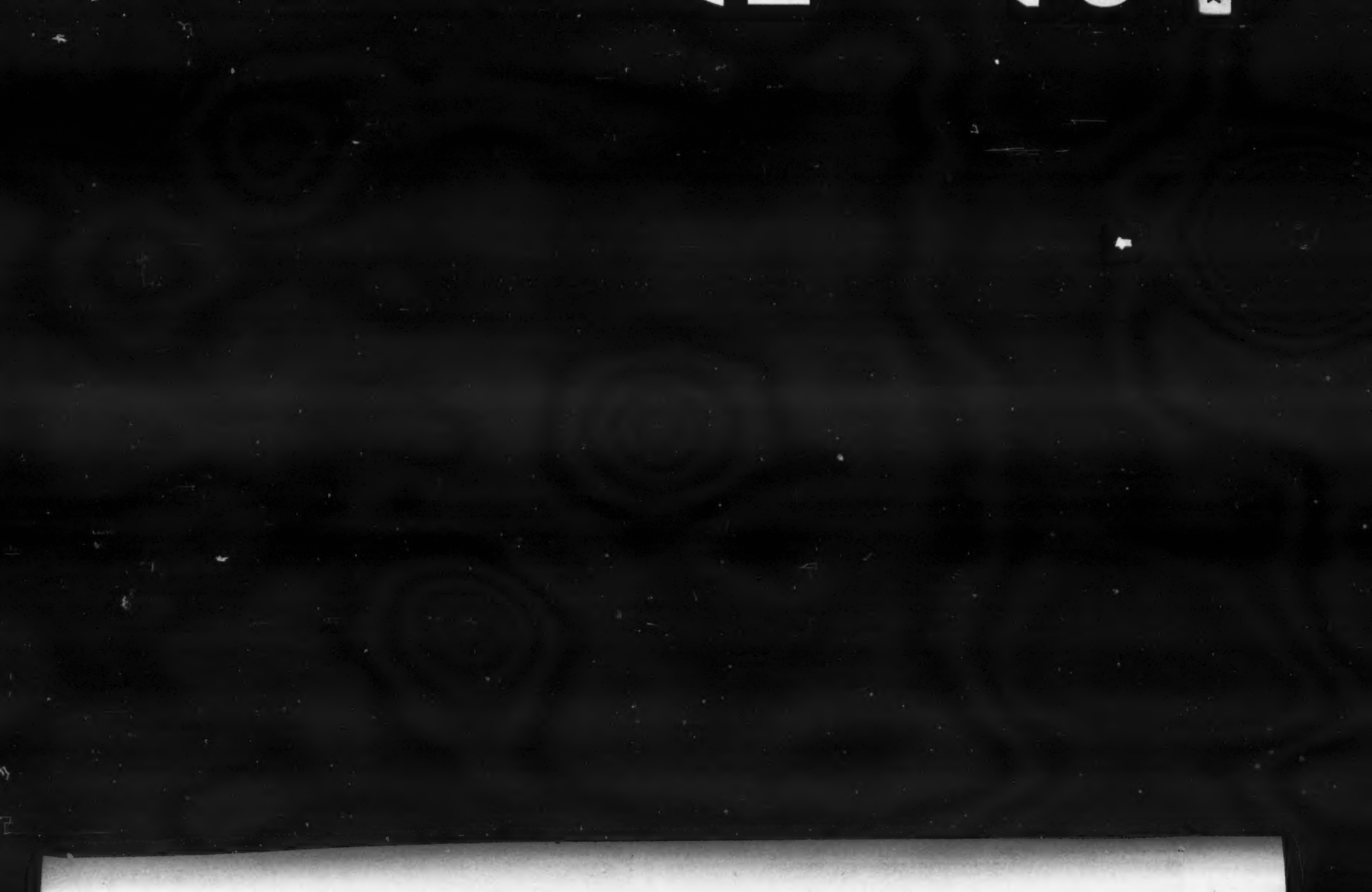


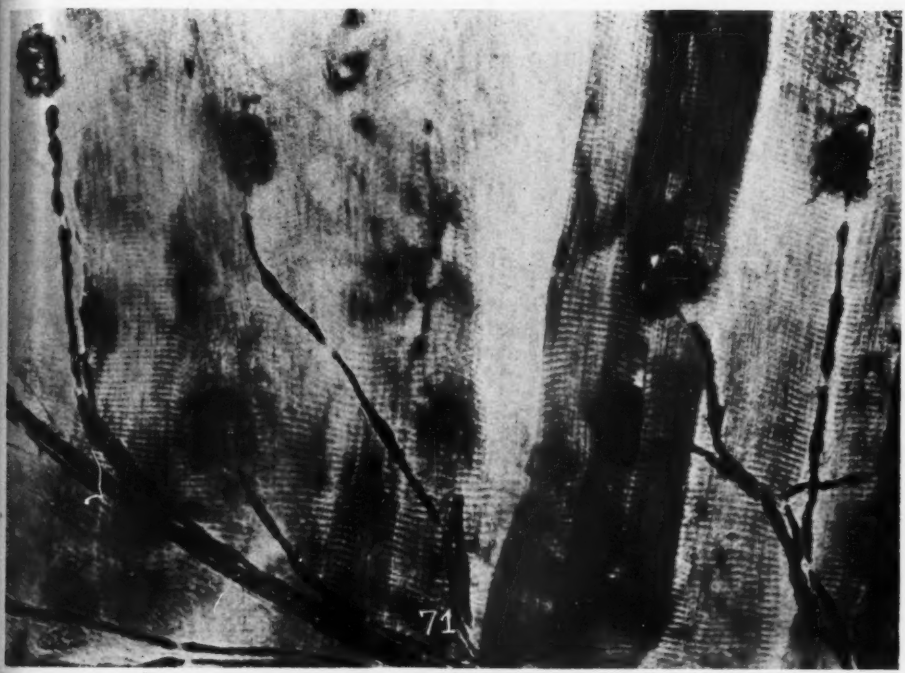


Carey, Massopust, Zeit, and Haushalter Motor End-Plates in Thermal Shock

Plate 52

Figs. 71 and 72. Sprays of axons of medullated nerve fibers of motor end-plates of the gastrocnemius muscle of the white rat, in the relatively normal state, excised 15 minutes after the intraperitoneal injection of nembutal. The variations in the structure of the relatively normal motor end-plates and muscle fibers (Fig. 71) are obvious. The retracted nerve endings are surrounded by a dense rim of Kühne's granules, blackened by impregnation with gold. In one location there is an irregular, halo-like space between the axons of the end-plate and the rim of Kühne's granules. The majority of the end-plates are expanded and have diminution or complete absence of Kühne's granules. The epilemmal axon, although irregularly beaded, is intensely impregnated with gold. The progressive dissolution of the motor end-plates (Fig. 72) is found 2 hours after scalding the skin for 10 seconds in water at 75° C. There is a progressive hypochrysophilia that terminates in complete achrysophilia of the motor end-plates during their progressive dissolution. The internal structure of the muscle fiber is composed of fine cross striations undergoing various stages of granulation. × 300.

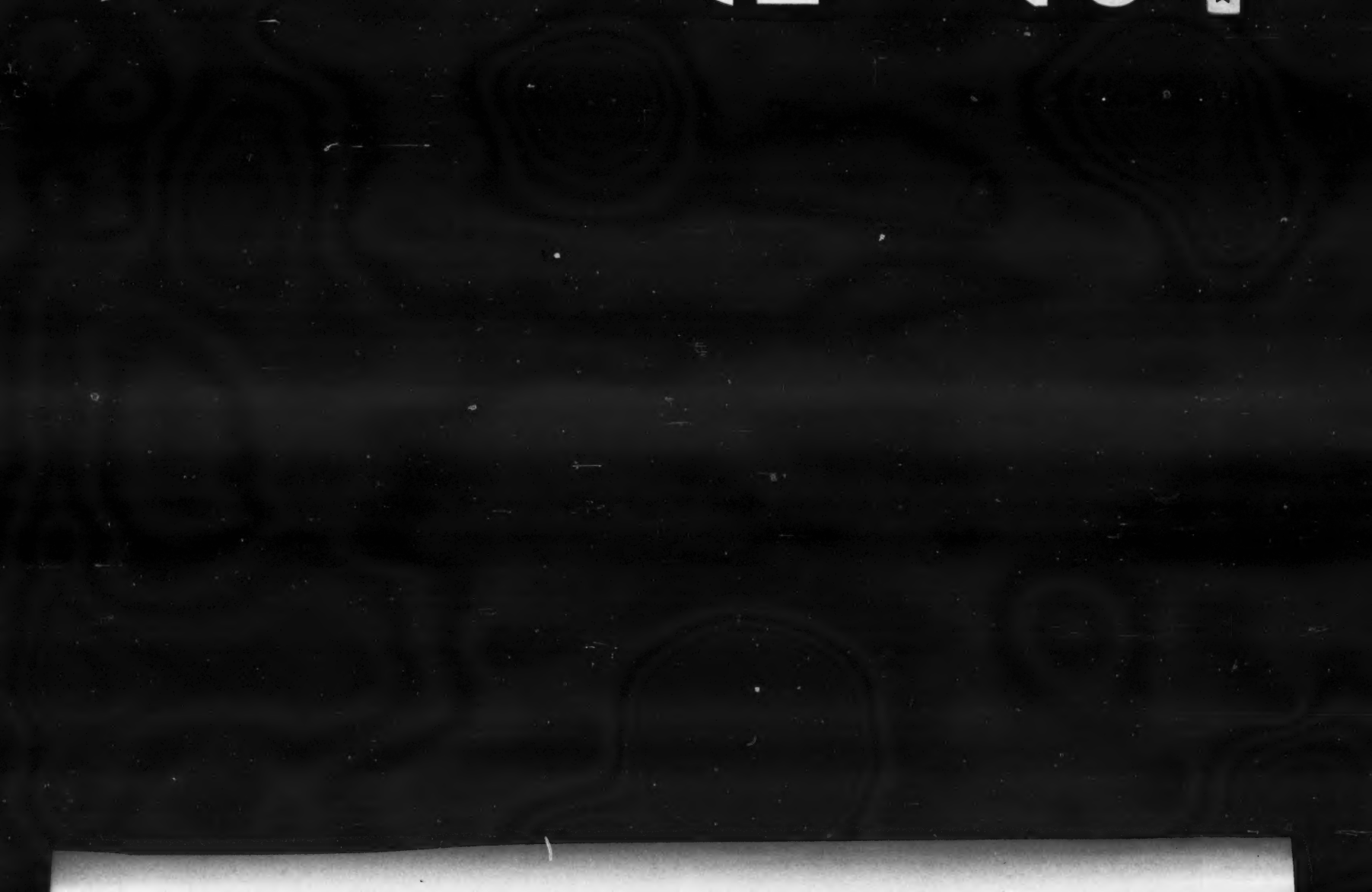


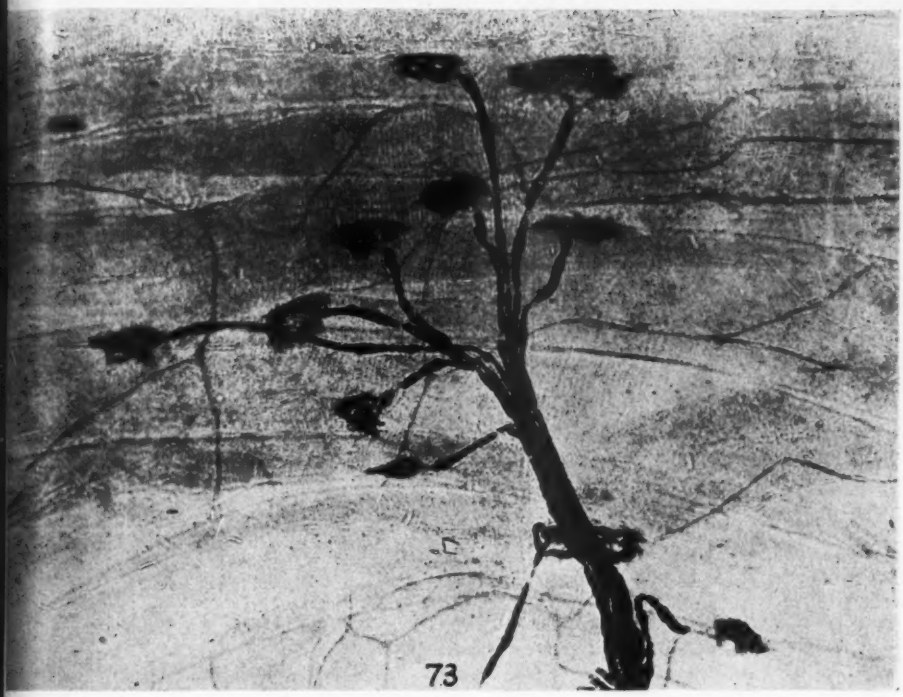

71

72

Plate 53

Figs. 73 and 74. Sprays of axons of medullated nerve fibers of motor end-plates of the gastrocnemius muscle of the white rat, in the relatively normal state, excised 15 minutes after the intraperitoneal injection of nembutal. There is a variation in the structure of the relatively normal motor end-plates (Fig. 73). The majority, however, are in a state of expansion, and the related muscle fibers are composed of exceedingly fine, closely spaced cross striations. The clearly defined epilemmal axons have an intense affinity for the impregnating gold. This normal morphologic state (Fig. 73) is in striking contrast to the motor end-plates and epilemmal axons from the gastrocnemius muscle (Fig. 74) excised 2 hours after the entire body of the rat (except the head and neck) had been immersed for 10 seconds in water at 75° C. There is a complete disappearance of the motor end-plates, the previous location of which is characterized by achrysophilia or absence of impregnation with gold. There is an irregular cluster of granules that occupies the location of the liquefied motor end-plates. There is a hypochrysophilia of the epilemmal axons. The depletion of these epilemmal axons of their gold-staining material extends in a centripetal direction away from the granular sites of the previous location of the motor end-plates. Exposure of the skin surface to water at a scalding temperature results in a thermal shock, part of the lesions of which are reflected in the neuromuscular apparatus. There is a denervation of the muscle fibers due to the structural disappearance of the motor end-plates and epilemmal axons. The study of the sequence of changes from the normal state to the stage of complete structural exhaustion reveals the secretory mechanism of the neuromuscular apparatus. × 300.

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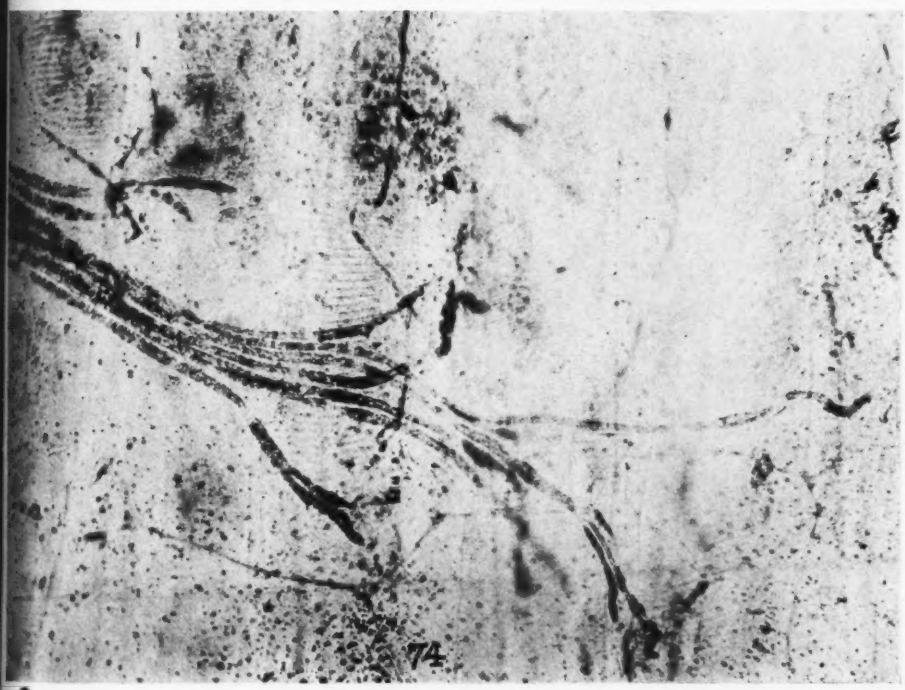
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